JOURNAL OF AGRICULTURAL RESEARCH

VOL. 63 WASHINGTON, D. C., OCTOBER 1, 1941 No. 7

INHERITANCE OF RUST REACTION IN A CROSS BETWEEN THE FLAX VARIETIES BUDA AND J. W. S.[1]

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INTRODUCTION

The discovery of physiologic specialization in *Melampsora lini* (Pers.) Lév. in North and South America and in Europe (*3, 4, 12, 13*)[2] has complicated the study of factors conditioning rust reaction in cultivated flax (*Linum usitatissimum* L.) as well as the development of rust-resistant varieties. On the basis of the reaction of 13 varieties of flax, 32 physiologic races have been differentiated, 24 from North and South America (*4*) and 8 from Europe (*12*). No variety is known to be immune from all the physiologic races, but 2 or more of the differential varieties are immune from any one race (*4*).

Henry (*9*) reported that an Argentine selection and the varieties Bombay and Ottawa 770B were immune from several collections of flax rust from North America and one from Europe. In a study of the inheritance of immunity from a North American collection of rust, in crosses involving these varieties, he found that immunity was conditioned by a single dominant factor in the varieties Bombay and Ottawa 770B and by either of two dominant factors in the Argentine selections. Myers (*10*) studied the inheritance of reaction to a field collection of flax rust and to a single physiologic race in 37 crosses involving 17 varieties or strains of flax. He found that reaction to both the collection and to the single physiologic race, except for minor differences probably attributable to environment, was similar and that he could account for the inheritance of immunity, near immunity, and resistance in the varieties he studied by assuming factors in two allelic series, L and M. L and M were dominant duplicate factors conditioning immunity; l^n and m^n were hypostatic to L and M and conditioned near immunity; and l^r and m^r were hypostatic to L and M and l^n and m^n and conditioned resistance. He was unable to interpret on a factorial basis segregations of crosses involving the varieties he designated as semiresistant, except by disregarding the difference between semiresistance and susceptibility in the progeny. With the exception of Bombay, which is susceptible to race 24, all the varieties studied by both Henry and Myers were susceptible to race 22.

No studies have been made in flax in which a variety resistant to one physiologic race of the rust fungus and susceptible to another

[1] Received for publication May 15, 1941. Cooperative investigations by the North Dakota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Italic numbers in parentheses refer to Literature Cited, p. 387.

race has been crossed with another variety reacting in a reverse manner to the two races. Such studies made with wheat varieties, involving resistance to *Puccinia graminis tritici* Eriks. and Henn., have shown that it is possible to obtain hybrids homozygous for resistance to both races, others homozygous for susceptibility to both races, and still others that react like the parent varieties (*6*, *7*, *8*, *11*). Aamodt (*1*), however, found that the factors for susceptibility, to physiologic race 1 of *P. graminis tritici* in Marquis, for moderate resistance in Kota, and for immunity in Kanred were allelic. Goulden, Neatby, and Welsh (*5*) concluded that the Marquis factors for high resistance to race 14 of *P. graminis tritici* were allelomorphic to the H–44–24 factors for moderate resistance to race 21, so that it was impossible to obtain a hybrid combining the resistance of both parents to these two races.

The original object of the present study was to determine the inheritance of factors conditioning rust reaction in a cross between two varieties of flax used to differentiate physiologic races of the flax rust fungus and known to react reversely with respect to immunity and susceptibility to physiologic races 4 and 7. The investigation was expanded to include a study of the inheritance of additional types of rust reaction involving physiologic races to which (1) both parents were immune; (2) one parent was immune and the other (a) resistant, (b) semiresistant, (c) moderately susceptible, or (d) susceptible; (3) one parent was susceptible and the other moderately susceptible; and (4) both parents were susceptible. The work was done at the North Dakota Agricultural Experiment Station, Fargo, N. Dak.

MATERIAL AND METHODS

Among the crosses made in the greenhouse in 1934, involving the varieties then being used to differentiate the physiologic races of *Melampsora lini*, was one between Buda (C. I.[3] 270–1) and J. W. S. (C. I. 708–1). Buda is susceptible to race 4 and immune from race 7, and J. W. S. is immune from race 4 and susceptible to race 7.

TABLE 1.—*Reaction of Buda and J. W. S. to 24 physiologic races of the flax rust fungus*

Variety	Reaction[1] to physiologic race—								
	20	1, 5, 6, 10, and 11	3, 14, 17, 18, and 23	2, 12, and 24	4, 8, 19, and 22	7	15	9 and 13	16 and 21
Buda	I	R+to R−	SR	S−	S	I	SR	S−	S
J. W. S.	I	I	I	I	I	S	S	S	S

[1] I=immune; R+=highly resistant; R=resistant; R−=moderately resistant; SR=semiresistant; S−=moderately susceptible; and S=susceptible.

The reaction of the two parental varieties is shown in table 1. The variety J. W. S. is either immune from or susceptible to each of the 24 physiologic races of the flax rust fungus that have been identified from North and South America. No difficulty has been experienced in determining its reaction even under adverse conditions for growth of flax. On the other hand, the reaction of Buda to different physio-

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

logic races ranges from immunity, through the intermediate stages, to susceptibility. Not only is the range of reaction of Buda extremely wide, but the intermediate reaction types are sensitive to environmental conditions, so that it is necessary to exercise caution in interpreting results. Environmental conditions that affect adversely the growth and vigor of the flax plant also depress rust development.

All phases of the tests were conducted in the greenhouse during the late fall, winter, and early spring of the years 1934 to 1939 inclusive. Insects were kept under control at all times, and no evidence of natural crossing between the flax varieties in the greenhouse was observed. Early in these investigations it became evident that consistent rust readings could be obtained only on vigorously growing plants of about the same age, grown under similar environmental conditions. Topsoil (Fargo clay) from the experiment station fields was found superior to numerous variations of topsoil, sand, and compost, and it was used exclusively in these trials. The same soil was used repeatedly throughout the year but was steamed for 2 hours at 15 pounds' pressure prior to each sowing. As the study progressed, it was found that flax seedlings growing under the low light intensities and short days that prevail during the winter months were more thrifty if the greenhouse temperature was relatively low and the soil rather dry. A night temperature of 55° to 60° F. and a day temperature of 60° to 70° were found to be most satisfactory. A 16-hour light day was maintained by supplemental illumination from 200-watt Mazda lamps during the period of pustule formation.

Preliminary tests had demonstrated that as many as five flax plants could be grown to maturity in a single 6-inch pot and that these plants would produce sufficient seed for a statistical analysis of the progenies. Three F_1 seeds were sown per pot and five F_2 seeds. Two sets of F_2 plants were included in these tests. Each F_2 plant grown during the winter of 1935–36 was inoculated with physiologic races 4 and 7. In addition, some of the F_2 plants in this series were subsequently inoculated with race 3, 13, 19, or 20. Those grown during the winter of 1936–37 were inoculated with physiologic races 3, 4, and 7. The two F_1 plants that supplied the F_2 seed used in 1936–37 were different from the plants that supplied the seed used the previous year. Reciprocal crosses were studied, but since the differences in the reaction of the progenies were not statistically significant, the data have been combined.

Except for minor variations, the methods of inoculation previously developed (*3*) for the physiologic-race investigations were used throughout these tests. The urediospore inoculum of each race was increased on a susceptible variety, and the spores were collected in glass vials and stored at 4° C. When the seedlings were 2 to 3 inches tall and had 6 to 10 leaves they were inoculated by dusting urediospores, with a camel's-hair brush, on the unfolding leaves of the terminal bud. The plants were placed in a moist chamber, sprayed, and incubated overnight (18 hours) and returned to the greenhouse bench. Pustules broke the epidermis of susceptible varieties about 10 days after inoculation. In these trials it was possible to test each plant with from two to five physiologic races by reinoculating the plants with different races at intervals of 7 to 8 days and picking off the leaves previously inoculated as soon as the rust had developed sufficiently to differentiate the type of pustule. The type of infection

produced on each plant was recorded and the plant tagged with colored yarn indicating a definite reaction to a specific race of rust.

As a result of severe rust infection, susceptible seedlings, if not killed directly, are weakened and often die before they mature seed. Since it was desired to obtain seed from each F_2 plant, care was taken to apply the inoculum to only a few leaves. These were less heavily inoculated than was customary in making physiologic-race determinations or in the subsequent tests of F_3 lines. Despite these precautions, some of the F_2 plants were so severely injured by the first test with a race to which they were susceptible that subsequent tests, with races to which they were resistant, were inaccurate. This difficulty was circumvented in the tests of the F_3 lines by using the races to which both parents were resistant in the earlier inoculations. The plants thus maintained a more normal growth, and complications due to reinfection were largely avoided.

Tests of F_3 lines were conducted in the same manner as were those of the F_2 lines except that eight plants were grown in a 4-inch pot, because it was usually not necessary to grow the plants to maturity. Four pots sown with eight seeds per pot formed the unit in testing F_3 lines. When doubt existed as to the accuracy or sufficiency of the test, additional units were sown if seed was available. In some cases checks on the accuracy of the F_3 rust readings were made by growing the F_3 plant to maturity and determining the rust reaction of the F_4 progeny.

For classifying rust reaction of flax plants, Myers (*10*) devised a key comprised of 11 classes, from 0 to 10 inclusive, based upon extent and type of infection. However, he was unable to follow it consistently in interpreting his results. For instance, seed from the Bison parent produced plants that ranged in reaction from class 2 to class 10 but gave no evidence of segregation. He stated that his class 4 and 5 reaction (semiresistant) was not very sharply differentiated genetically from the susceptible. To give a factorial interpretation of his results, he found it necessary to group classes 5 to 10 or 6 to 10 together as susceptible and classes 1, 2, and 3 as resistant. The inclusion of extent of infection in his classification appears to have accentuated difficulties in classifying the reaction of the individual plants grown under greenhouse conditions.

The following key to classes of host reaction and infection types as used in differentiating physiologic races of *Melampsora lini* (*3*) served as a guide for classifying the rust reaction of the individual plants in the present tests.

Classes of host reaction:	*Types of rust infection*
Nearly immune	(0) No uredia developed; hypersensitive flecks or necrotic lesions usually present, but sometimes there is no evidence of infection.
Resistant	(1) Uredia minute to small, rarely extending through the leaf, usually distinct and scattered in chlorotic to necrotic areas, but in some cases pustule formation is not accompanied by either chlorosis or necrosis of the surrounding leaf tissue.
	(2) Uredia small to medium, associated with distinct necrosis of the leaf; may be scattered or may form crustlike aggregations in necrotic areas; if isolated, usually are surrounded by a necrotic zone.

Classes of host reaction—Continued.		*Types of rust infection*
Semiresistant	(3—)	Uredia variable; heavily inoculated areas necrotic, with arrested pustule development; medium to large pustules produced in healthy tissue adjacent to necrotic areas; pustules on stem and cotyledons small but with no evidence of hypersensitiveness.
Moderately susceptible	(3)	Uredia medium to large; well-developed but not compound; usually extending through the leaf to both surfaces; development somewhat retarded on older leaves and in heavily infected portions of the younger leaves; tissues adjacent to uredia may become more or less chlorotic as the pustules mature.
Susceptible	(4)	Uredia large and, if isolated, usually compound, extending through leaf to both surfaces; at first leaves show little chlorosis but later may become chlorotic and die prematurely.

In this key, the reaction class is determined by the type of pustule. In the subdivisions of the classes of host reaction, such as highly resistant, moderately resistant, moderately susceptible, and susceptible, consideration is given to the extent of infection as manifested by the relative number, size, and sporulation of pustules.

EXPERIMENTAL RESULTS

REACTION OF F_1 PLANTS AND PROGENY OF PARENT PLANTS

The F_1 plants of Buda × J. W. S. were immune from races 4 and 7 of *Melampsora lini*, indicating that the factors conditioning immunity were dominant in these varieties, as well as those studied by Henry (*9*) and Myers (*10*). Seed from the parent plants used in making the crosses also was tested. That from the Buda parent was homozygous for susceptibility to race 4 and for immunity from race 7, while that from the J. W. S. parent was homozygous for immunity from race 4 and for susceptibility to race 7.

REACTION OF F_2 PLANTS TO RACES 4 AND 7

The F_2 plants grown in the greenhouse during the winter of 1935–36 are classified in table 2 according to their reaction to races 4 and 7

TABLE 2.—*Reaction of F_2 plants of Buda × J. W. S. to physiologic races 4 and 7 of the flax rust fungus*

Reaction of F_2 plants to race 4 [1]	F_2 plants showing indicated reaction to race 7 [2 3]			
	Immune	Resistant	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune	209	18	33	260
Susceptible	88	0	0	88
Total	297	18	33	348

[1] χ^2 for 3 immune : 1 susceptible to race 4=0.0153; P=0.90 to 0.95.
[2] χ^2 for 3 immune : 1 not immune from race 7=19.86; P <0.01.
[3] χ^2 for 15 resistant and immune : 1 susceptible to race 7=6.207; P <0.01.

of *Melampsora lini*. Results obtained from tests with races 3, 13, 19, and 20 were not sufficiently informative to warrant tabulation.

Each of 165 F_2 plants tested was immune from race 20. All plants tested reacted the same to race 19 as to race 4. Tests with races 3 and 13 were not satisfactory, as the plants had become too old to consistently differentiate intermediate infection types.

A single dominant factor conditions immunity from race 4 in this cross as the segregation of 260 immune to 88 susceptible F_2 plants approximates a 3 to 1 ratio, P lying between 0.90 and 0.95. Apparently, a modifying factor was involved in the reaction to race 7, as some plants were distinctly resistant to this race, while others were either immune or susceptible. Even if all plants not immune from race 7 were considered as a unit, for a ratio of 3 immune to 1 not immune P was less than 0.01. This suggested that the test of the F_2 plants for reaction to race 7 was not accurate, or that reaction to this race was conditioned by more than one factor.

TESTS OF F_3 LINES WITH RACES 4 AND 7

The 348 F_3 lines of Buda × J. W. S. are classified in table 3 according to the reaction of the progenies to races 4 and 7 of *Melampsora lini*. The 88 F_2 plants that were susceptible to race 4 and immune from race 7 yielded F_3 progenies homozygous for the same reaction. This confirmed the accuracy of the test of the F_2 plants for their reaction to race 4. Of the 260 lines descended from F_2 plants immune from race 4, 175 segregated for susceptible and immune plants and 85 were homozygous for immunity to that race. The fit of the observed ratio to the theoretical 1:2:1 for reaction of the 348 F_3 lines to race 4 was close, P lying between 0.95 and 0.98.

TABLE 3.—*Reaction* [1] *of F_3 lines of Buda × J. W. S. to physiologic races 4 and 7 of the flax rust fungus*

Parental F_2 plants			F_3 progenies having indicated reaction to races 4 and 7						
Number	Reaction to race—		All I (race 4)			I and S (race 4)			All S (race 4)
	4	7	All R (race 7)	R, SR, S (race 7)	All S (race 7)	I and R (race 7)	I, R, SR, S (race 7)	I and S (race 7)	All I (race 7)
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
209	I	I	21	13	.....	57	77	41	.....
18	I	R	.....	18	.....	.....	.....	.....	.....
33	I	S	.....	6	27	.....	.....	.....	.....
88	S	I	.....	.....	.....	.....	.....	.....	88
Total observed [2]			21	37	27	57	77	41	88
Expected, 1:2:1:2:4:2:4			21.75	43.5	21.75	43.5	87	43.5	87
Observed [3]			21	37	27	134		41	88
Expected, 1:2:1:6:2:4			21.75	43.5	21.75	130.5		43.5	87
Observed [4]			85			175			88
Expected, 1:2:1			87			174			87

[1] See footnote 1, table 1.
[2] χ^2=7.755; P=0.20 to 0.30.
[3] χ^2=2.513; P=0.70 to 0.80.
[4] χ^2=0.063; P=0.95 to 0.98.

The progeny of each of the 51 F_2 plants that had not been immune from race 7 was homozygous for immunity from race 4 and for some degree of susceptibility to race 7. In addition, the progenies of 34

of the 209 F_2 plants that had been classed as immune from both races 4 and 7 were immune from race 4 and were either homozygous for resistance or segregated for resistance, semiresistance, and susceptibility to race 7. These 34 F_2 plants had resisted infection probably because of the use of insufficient inoculum and because of the adverse effect on the vigor of the host of low light intensity during a prolonged cold period when the glass of the greenhouse was continuously coated with frost. The progenies of 6 of the 33 F_2 plants that had been classed as susceptible to race 7 segregated for susceptible and resistant to semiresistant plants. Apparently the factor conditioning resistance to race 7 in this cross was not completely dominant and was sensitive to environment, as the reaction of heterozygous plants sometimes approached the moderately susceptible. Of the 85 F_3 lines that were homozygous for immunity from race 4, 27 were homozygous for susceptibility, 37 segregated for susceptible, semiresistant, and resistant plants, and 21 were pure for resistance to race 7.

The 175 F_3 lines heterozygous for reaction to race 4 were heterozygous also for reaction to race 7. All plants of 41 lines were either susceptible to one race and immune from the other or immune from both. Each of the remaining 134 lines contained plants having a distinctly resistant reaction to race 7. In 77 of these lines one or more plants were highly susceptible to race 7, and in the remaining 57 lines all plants were either resistant or immune. Since each of the 10,540 F_3 plants as well as each of the 348 F_2 plants tested was immune from either race 4 or race 7 or from both, the factors conditioning immunity from race 4 in J.W.S. and from race 7 in Buda apparently were in one allelic series.

These results suggest that Buda also carries a factor for resistance to race 7, independent of and hypostatic to the immune factor. If this hypothesis is correct, the ratio of F_3 lines for reaction to race 7 would approximate 1 resistant to 2 resistant and susceptible to 1 susceptible to 2 immune and resistant to 4 immune, resistant, and susceptible to 2 immune and susceptible to 4 immune.

The fit of the observed ratio to the theoretical was good (table 3), P lying between 0.20 and 0.30. The greatest deviation from the theoretical distribution was in groups heterozygous to race 4 and containing plants having a resistant reaction to race 7. There were too many lines containing only resistant and immune plants and too few having susceptible, resistant, and immune plants. The criterion for separation of these 2 groups was the occurrence of plants susceptible to race 7. In a sample of 32 plants the theoretical distribution for reaction to race 7 in one group was 8 resistant to 24 immune and in the other, 2 susceptible to 6 resistant to 24 immune. The theoretical expectation of obtaining no susceptible plant in a random sample of 32 from the latter group is 0.134. Therefore, it is probable that approximately 11 of the 57 F_3 lines that had only resistant and immune plants when tested with race 7 would have had susceptible individuals had a larger sample been used. This is indicated by the close approach to the theoretical ratio of F_3 lines when these 2 groups are combined. P for a 1:2:1:6:2:4 distribution lies between 0.70 and 0.80.

REACTION OF F_2 PLANTS TO RACES 3, 4, AND 7

Tests of some of the F_3 lines susceptible to race 4 indicated a segregation for resistance and susceptibility to race 3. As adequate seed

for additional tests of a number of the F_3 lines was not available, a second series of infection studies was made in which races 3, 4, and 7 were used on 237 F_2 plants. The results are shown in table 4.

As in the first series of infection studies, the fit of the F_2 plants to a ratio of 3 immune to 1 susceptible to race 4 was good, P lying between 0.30 and 0.50. The inoculation with race 4 preceded that with race 3, and as a consequence all plants in any degree susceptible to the latter race had been weakened by the attack of race 4 and the subsequent stripping of infected leaves. This fact, together with the increased age of the plants at the time of inoculation and the adverse climatic conditions that prevailed during the period that race 3 developed in the plants, obscured differences in infection types. The segregation for intermediate reaction to race 3 was indefinite, but all plants that had been susceptible to race 4 were attacked in varying degrees of severity by race 3, and all that had been immune from race 4 were also immune from race 3.

The test of the F_2 plants with race 7, although not wholly satisfactory, was better than the earlier one. The fit for a ratio of 15 immune and resistant to 1 susceptible was satisfactory, P lying between 0.30 and 0.50. However, P for a ratio of 12 immune to 3 resistant to 1 susceptible was less than 0.01. Unless the infection is severe enough to stunt or kill susceptible plants, some that are intermediate in reaction to race 7 apparently escape or resist infection.

TABLE 4.—*Correlation table showing reaction of F_2 plants of Buda × J. W. S. to physiologic races 3, 4, and 7 of the flax rust fungus*

F_2 plants having indicated reaction [1] to—					
Race 3	Race 4 [2]	Race 7 [3]			
		I	SR to R	S	Total
		Number	*Number*	*Number*	*Number*
I	I	140	15	18	173
R– to S–	S	28	--	--	28
S	S	36	--	--	36
Total		204	15	18	237

[1] See footnote 1, table 1.
[2] χ^2 for 3 I : 1 S to race 4=0.508; P=0.30 to 0.50.
[3] χ^2 for 15 I and SR to R : 1 S to race 7=0.612; P=0.30 to 0.50.

TESTS OF F_3 LINES WITH RACES 3, 4, AND 7

The rust reaction of 26 to 157 plants from each of the 236 F_3 lines was determined by successive inoculation with races 3, 4, and 7 as previously outlined. Adverse growing conditions, especially during January and February, interfered with the accurate reading of intermediate reaction to races 3 and 7. Later, during the spring months, when conditions in the greenhouse were more favorable for growth of the host and for rust development, tests of lines having plants of doubtful reactions were repeated, and satisfactory results were obtained. When grouped according to the diversity of reaction of each F_3 progeny to races 3, 4, and 7, the 236 F_3 lines fall into the 9 classes listed in table 5.

TABLE 5.—*Reaction* [1] *of F_3 lines of Buda × J. W. S. to physiologic races 3, 4, and 7 of the flax rust fungus*

Reaction of F_2 plants to—			F_3 lines	F_3 plants	Reaction of F_3 plants to—		
Race 3	Race 4	Race 7			Race 3	Race 4	Race 7
			Number	*Number*			
S, SR, R	S	I	22	910	SR	S	I
S, SR, R	S	I	27	258	S	S	I
				856	SR	S	I
S, SR	S	I	15	616	S	S	I
Total			64	2,640			
I	I	I	25	298	SR	S	I
				637	I	I	I
				273	I	I	R
I	I	I	66	193	S	S	I
				630	SR	S	I
				1,904	I	I	I
				583	I	I	R
				229	I	I	S
I	I	I	32	622	S	S	I
				1,286	I	I	I
				667	I	I	S
Total			123	7,322			
I	I	I, R–	11	373	I	I	R
I	I	SR, R–, I	21	1,004	I	I	R
				320	I	I	S
I	I	S	17	982	I	I	S
Total			49	2,679			
Grand total			236	12,641			

[1] See footnote 1, table 1.

None of the 12,641 plants in this test was susceptible both to race 7 and to either of races 3 or 4. This fact, as well as the behavior of the 348 F_3 lines as presented in table 3, substantiates the conclusion that the factors conditioning immunity in this cross are dominant and allelic. Of the 236 F_3 lines tested, 64 were homozygous for susceptibility to race 4 and for immunity from race 7, 123 were heterozygous, having plants immune from both races, and 49 were homozygous for immunity from race 4 and for some degree of susceptibility to race 7. This observed ratio fits satisfactorily the theoretical 1 : 2 : 1, *P* lying between 0.30 and 0.50. The reaction of the F_3 population in the 3 groups of lines having characteristic reaction to race 4 indicates that the semiresistant reaction to race 3 and the resistant reaction to race 7 are incompletely dominant to susceptibility (fig. 1, *A, a, b; B, a, b*) and are conditioned by factors independent of and hypostatic to the factors conditioning immunity. Of the 64 lines susceptible to race 4 and immune from race 7, 15 were homozygous for susceptibility to race 3; 27 were heterozygous, having both semiresistant and susceptible plants; and 22 were homozygous for semiresistance. The *P* for fit of this distribution to the theoretical 1 : 2 : 1 lay between 0.20 and 0.30. Of the 123 lines heterozygous for immunity, 25 were homozygous for the resistant factors, as all plants not immune from races 3, 4, and 7 were either semiresistant to race 3 and immune from race 7 or resistant to race 7 and immune from race 3; 66 were heterozygous for the resistant factors; and 32 were homozygous for the recessive susceptibility factors, as all plants not immune from both races 3 and 7 were either susceptible to race 3 and

immune from race 7 or susceptible to race 7 and immune from race 3. The fit of the observed ratio of F_3 lines to the expected 1 : 2 : 1 for segregation of the resistant factor in this heterozygous immune group was good, *P* lying between 0.30 and 0.50. Of the 49 F_3 lines immune from races 3 and 4, 11 were homozygous for resistance to race 7; 21 were heterozygous, having both susceptible and resistant plants; and 17 were homozygous for susceptibility. The fit of the observed ratio to the calculated 1 : 2 : 1 is satisfactory, *P* lying between 0.20 and 0.30.

Myers (*10*) concluded from his studies that a single dominant factor conditioned immunity from a collection of rust and from race 4 in Ottawa 770B and that C. I. 438 carried the same factor for immunity as Ottawa 770B. However, C. I. 438 is susceptible to and Ottawa

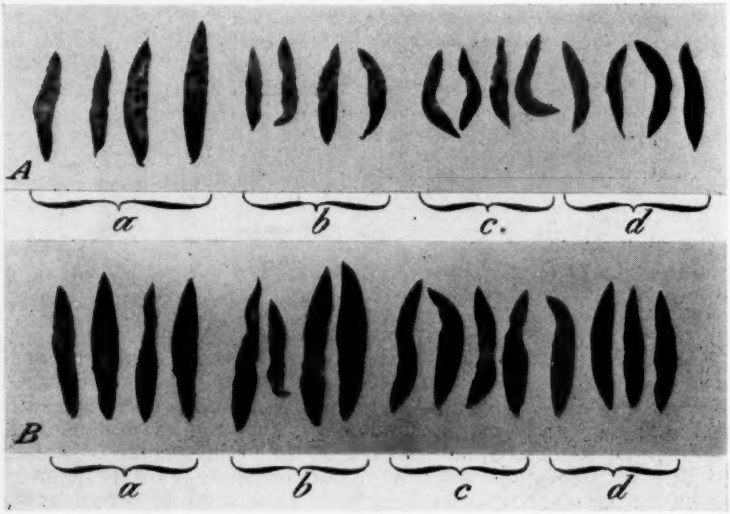


FIGURE 1.—Reaction of homozygous and heterozygous F_3 segregates from the hybrid Buda × J. W. S. to physiologic races 3 and 7, showing the dominance of the immune factors L^1 and L^2 and the incomplete dominance of the resistant factor *R*. *A*, Race 3: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; and *d*, L^1L^2rr. *B*, Race 7: *a*, L^2L^2rr; *b*, L^2L^2Rr; *c*, L^2L^2RR; and *d*, L^1L^2rr. Natural size.

770B is immune from races 19 and 20; so it is probable that the factors conditioning immunity in these 2 varieties are allelic rather than identical. Both Ottawa 770B and C. I. 438 were immune from the strains of rust used by Myers, so that this difference in reaction of the varieties was not discernible in his tests. Preliminary studies of the writer have indicated that the factor conditioning immunity from 18 races, carried by J. W. S., is allelic to the factor of Ottawa 770B conditioning immunity from 23 races. Myers designated the pair of immunity factors of Ottawa 770B as *LL*. As indicated in table 6, it is proposed to designate the factors conditioning immunity from race 7 in Buda as L^1 L^1 and the factors conditioning immunity from race 4 in J. W. S. as L^2 L^2. The relation of the factors conditioning semi-resistance to race 3, carried by Buda, to Myers' *MM* series of allels conditioning rust reaction is not known. Until this relation is de-

termined, it is proposed to designate the semiresistant factors carried by Buda as *RR* and the recessive allels in J. W. S. as *rr*. On the basis of this hypothesis, the F_3 lines are grouped in table 6 according to the F_2 genotypes, as indicated by the reaction of the F_3 population to races 3, 4, and 7. Values of χ^2 for fit to the expected ratios for each of the segregating lines were calculated, and the value of P was determined for each. Of the 171 F_3 lines segregating for reaction to races 3 or 7, the P for 1 line lay between 0.01 and 0.02, the P for 5 lines lay between 0.02 and 0.05, and the P for 165 lines was greater than 0.05. The formula given by Fisher (*2*), $\sqrt{2\chi^2}-\sqrt{2n-1}$, when applied to the sum of the χ^2 values of the 171 segregating lines, gave a normal deviate of -1.478 ± 1, showing that the data are in accord with the hypothesis.

TABLE 6.—*Segregation for F_2 genotypes in Buda × J. W. S. as indicated by the reaction of F_3 lines to physiologic races of the flax rust fungus*

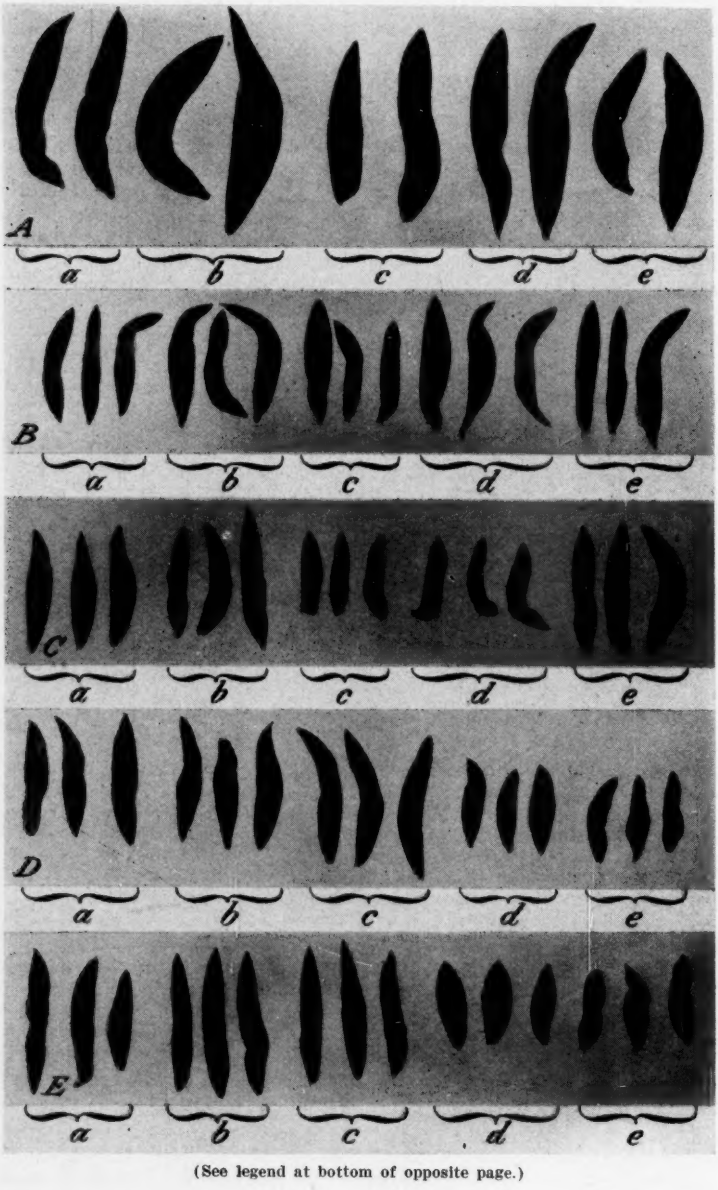
Variety or hybrid	F_2 genotype	Theoretical ratio	F_3 lines segregating		Ratio of F_3 plants	Reaction [2] of F_3 plants to—		
			Expected	Observed [1]		Race 3	Race 4	Race 7
			Number	*Number*				
Buda					All	SR	S	I
J. W. S.					All	I	I	S
F_2	L^1L^1RR	1	14.8	22	All	SR	S	I
	L^1L^1Rr	2	29.6	27	1	S	S	I
					3	SR	S	I
	L^1L^1rr	1	14.8	15	All	S	S	I
	L^1L^2RR	2	29.6	25	1	SR	S	I
					2	I	I	I
	L^1L^2Rr	4	59.3	66	1	I	I	R
					1	S	S	I
					3	SR	S	I
					8	I	I	I
					3	I	I	R
					1	I	I	S
					1	S	S	I
	L^1L^2rr	2	29.6	32	2	I	I	I
					1	I	I	S
	L^2L^2RR	1	14.8	11	All	I	I	R
	L^2L^2Rr	2	29.6	21	3	I	I	R
					1	I	I	S
	L^2L^2rr	1	14.8	17	All	I	I	S

[1] $\chi^2=9.252$; $P=0.30$ to 0.50.
[2] See footnote 1, table 1.

In table 6 is recorded the number of F_3 lines descended from each of the theoretical F_2 genotypes, as indicated by the reaction of the F_3 population to physiologic races 3, 4, and 7, and the expected distribution of F_3 plants from each F_2 genotype for reaction to these races. The fit of the F_3 lines to a 1:2:1:2:4:2:1:2:1 distribution was good. For the 236 lines, P lay between 0.30 and 0.50, indicating that the data are in agreement with the hypothesis.

REACTION OF F_3 GENOTYPES TO RACES DIFFERENTIATED BY BUDA AND J. W. S.

The correlation of reaction to various races of the flax rust fungus was studied by inoculating plants of F_3 lines, whose reaction had been successively determined to races 3, 4, and 7, with one or more of the following races: 1, 2, 5, 8, 9, 10, 16, 19, 20, 21, and 22. Although a wide range of reaction combinations was included in these tests, there was no indication of the presence of factors conditioning rust



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reaction other than those affecting races 3, 4, and 7. Since the age of the plant influences the expression of the intermediate infection types, these supplemental tests on 40- to 60-day-old plants were not strictly comparable with those of races 3, 4, and 7 on 20- to 30-day-old plants. To obviate this, a line was selected for study from each of the four genetically different groups that were homozygous for factors conditioning rust reaction, as indicated by the tests with races 3 and 7. Line 7, L^1L^1RR, was similar to the Buda parent and line 8, L^1L^1rr, possessed the factors conditioning immunity from race 7 but lacked the factors conditioning semiresistance to race 3. Line 575, L^2L^2rr, possessed the factors conditioning immunity from race 4 and susceptibility to race 7 of the J. W. S. parent, and line 584, L^2L^2RR, had in addition to the immune factors of the J. W. S. parent the factors that conditioned semiresistance to race 3 in Buda. The reaction of the parents and of these four F_3 lines to a race representative of each group of races differentiated by the reaction of Buda and J. W. S. is given in table 7.

TABLE 7.—*Seedling reaction to 9 physiologic races of the flax rust fungus of Buda, J. W. S., and F_3 lines of Buda × J. W. S. homozygous for factors conditioning rust reaction*

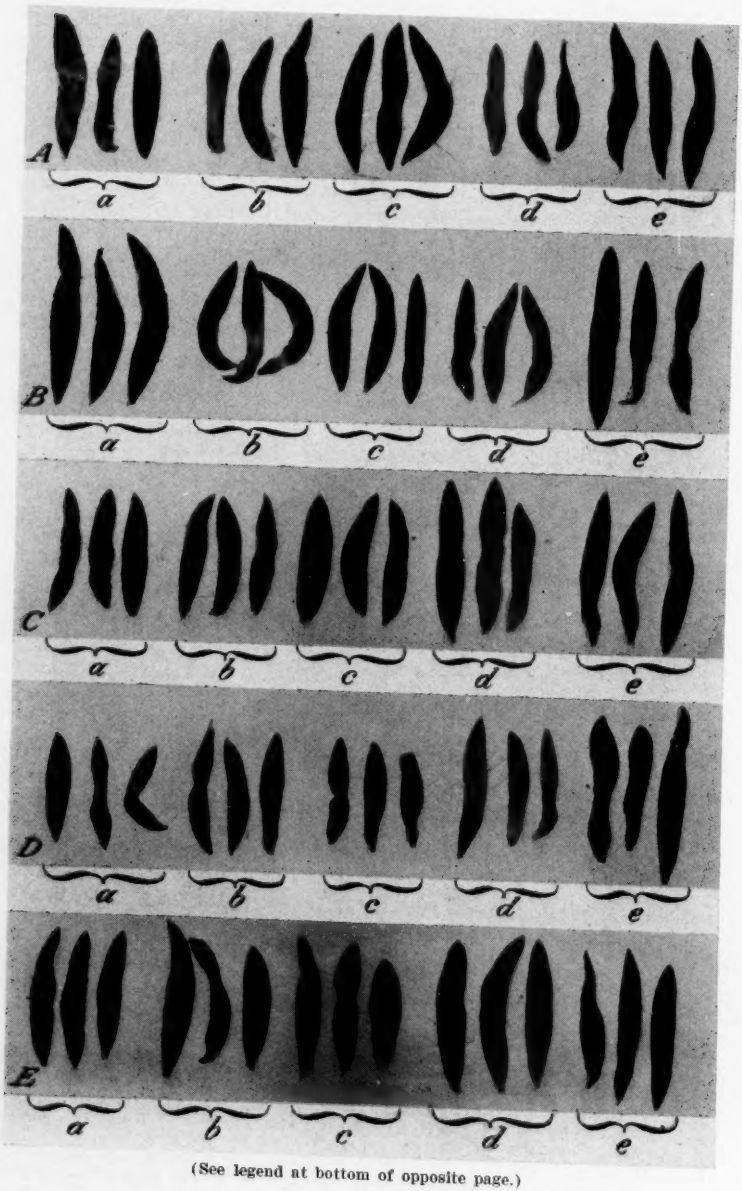
Variety or F_3 line	Genotype	Seedling reaction [1] to physiologic race—								
		20	5	1	3	2	4	7	9	16
Buda	L^1L^1RR	I	R+	R+ to R−	SR	S−	S	I	S−	S
J. W. S.	L^2L^2rr	I	I	I	I	I	I	S	S	S
F_3 line:										
7	L^1L^1RR	I	R+	R+ to R−	SR	S−	S	I	S−	S
8	L^1L^1rr	I	R to R−	R to S—	S	S	S	I	S− to S	S
575	L^2L^2rr	I	I	I	I	I	I	S	S	S
584	L^1L^1RR	I	I	I	I	I	I	R	S− to S	S

[1] See footnote 1, table 1.

REACTION TO RACE 20

Both parent varieties, Buda and J. W. S., have been consistently immune from race 20. During these studies 165 F_2 plants as well as numerous F_3 lines, including lines heterozygous as well as those homozygous for factors conditioning rust reaction, were tested and were invariably immune. However, the immune reaction of plants having the J. W. S. factor for immunity (L^2L^2) was not identical with that of plants having the Buda factor (L^1L^1) and was affected by environmental conditions. Plants having the immune factors of the J. W. S. parent tested in February, when light is deficient, developed distinct necrotic flecks and a tendency toward chlorosis in heavily inoculated portions of the leaves (fig. 2, *A*, *a*, *c*, *e*). In similar tests, conducted during the latter part of March under more

FIGURE 2.—Reaction of the parent varieties Buda (L^1L^1RR) and J. W. S. (L^2L^2rr) and F_3 hybrid segregates of indicated genotype to physiologic races of *Melampsora lini* differentiated by Buda and J. W. S. *A*, Race 20, reaction in midwinter: *a*, J. W. S.; *b*, Buda; *c*, L^2L^2RR; *d*, L^1L^1rr; and *e*, L^2L^2rr. *B*, Race 20, reaction in spring: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *C*, Race 5: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *D*, Race 1: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *E*, Race 17: *a*, L^1L^1rr; *b*, L^1L^1Rr; *c*, L^1L^1RR; *d*, Buda; and *e*, J. W. S. *A*, × 1. 5; *B* to *E*, natural size.



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favorable growing conditions, necrotic lesions were absent and the principal evidences of inoculation were the distortion and slight stunting of heavily inoculated leaves and the gray mycelial weft left by the germ tubes (fig. 2, *B*, *c*, *e*). In the February tests, the response to inoculation with race 20 of plants having the Buda factor for immunity was a slight flecking and distortion of the heavily inoculated portions of the leaves (fig. 2, *A*, *b*, *d*), but in tests during the latter part of March the leaves became distorted and developed chlorotic to necrotic lesions (fig. 2, *B*, *a*, *b*, *d*). Although no rudimentary uredia have been observed in these chlorotic to necrotic areas, this response was suggestive of the reaction of Buda to race 5 (fig. 2, *C*, *d*). If failure to form uredia is the criterion for immunity from rust, then immunity is dependent not on exclusion of the pathogen but on the limitation of its development. It is not improbable that, under favorable environmental conditions, the development of race 20 might be extended to permit the formation of rudimentary uredia in these chlorotic to necrotic areas.

Reaction to Races 1 and 5

Race 1 is a composite of races of restricted pathogenicity as measured by ability to attack the rust-differentiating varieties (*3*). The reaction of Buda to the different strains of race 1 varies from high resistance through intermediate types to moderate resistance. The strains to which Buda is highly resistant (*R*+) appear to be distinct from those to which it is moderately resistant (*R*−), but their separation is complicated by the occurrence of intermediate strains, so that impracticably rigid control of environmental conditions is required to secure consistent differentiation.

Immunity from all strains of race 1 and from race 5 is conditioned by the L^2 factor of J. W. S. (fig. 2, *C*, *e*; *D*, *e*). Both factors that condition rust reaction in Buda are operative to races 1 and 5. Line 8, L^1L^1rr, was resistant to race 5 (fig. 2, *C*, *a*) and to strains of race 1 to which Buda was highly resistant, and was moderately susceptible (fig. 2, *D*, *a*) to the strains of race 1 to which Buda was moderately resistant. Line 7, L^1L^1RR, was more resistant to races 5 (fig. 2, *C*, *c*) and 1 (fig. 2, *D*, *c*) than was line 8; and heterozygous plants, L^1L^1Rr, were intermediate in reaction (fig. 2, *C*, *b*; *D*, *b*).

Reaction to Races 3 and 17

The semiresistant reaction of Buda differentiates races 3 and 17 from races 5 and 1, respectively. Line 8, L^1L^1rr, was susceptible both to race 3 (fig. 3, *A*, *a*) and to race 17 (fig. 2, *E*, *a*). However, the pustules of the latter race, although more numerous, were smaller, and the infected leaves tended to die prematurely, indicating that

Figure 3.—Reaction of the parent varieties Buda (L^1L^1RR) and J. W. S. (L^2L^2rr) and F_3 hybrid segregates of indicated genotype to physiologic races of *Melampsora lini* differentiated by Buda and J. W. S. *A*, Race 3: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *B*, Race 7: *a*, L^2L^2rr; *b*, L^2L^2RR; *c*, L^1L^1rr; *d*, Buda; and *e*, J. W. S. *C*, Race 4: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *D*, Race 9: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. *E*, Race 16: *a*, L^1L^1rr; *b*, L^1L^1RR; *c*, L^2L^2RR; *d*, Buda; and *e*, J. W. S. Natural size.

there was some degree of incompatibility between host and pathogen and that the L^1L^1 factors, while ineffective to race 3, were not wholly so to race 17. The reaction of line 7, L^1L^1RR, and plants heterozygous for the resistant factor, L^1L^1Rr, to races 3 and 17 also varied. Isolated pustules of race 3 were large and the copiously inoculated regions of the leaves became rather sharply necrotic (fig. 3, *A*, *b*, *d*). The pustules of race 17 were small and numerous, and the well-inoculated portions of the leaves (fig. 2, *E*, *b*, *c*, *d*), although dying prematurely, were characterized by a chlorosis rather than the sharp necrosis of race 3.

Reaction to Race 2

The reaction of F_3 lines of various genotypes to race 2 was similar to their reaction to race 3, except that genotype L^1L^1RR (line 7) was semiresistant to race 3 and moderately susceptible to race 2.

Reaction to Races 4, 8, 19, and 22

Extensive tests of F_2 plants and F_3 lines were made with races 4, 8, 19, and 22, to which Buda is susceptible and J. W. S. immune. Immunity from these races was dominant and was conditioned by the factor L^2 (fig. 3, *C*, *c*, *e*). The susceptibility of seedlings of Buda and of F_3 line 7, L^1L^1RR (fig. 3, *C*, *b*, *d*), shows that neither the L^1 nor the R factors condition resistance to these races. However, when infected by races 4 or 8, older plants of F_3 line 7, especially when grown under adverse conditions, had fewer and smaller pustules and showed a greater tendency toward chlorosis and premature death of infected leaves than did plants of F_3 line 8, L^1L^1rr (fig. 3, *C*, *a*). All plants not having the immune factor L^2 were highly susceptible to races 19 and 22, even when grown under adverse conditions.

Reaction to Race 7

Buda is immune from and J. W. S. susceptible to race 7 (fig. 3, *B*, *d*, *e*), the reverse of the reaction of these parent varieties to races 4, 8, 19, and 22. Immunity from race 7 was conditioned by the dominant factor L^1 (fig. 3, *B*, *c*, *d*). The factors RR, which condition semiresistance to race 3 in Buda, condition resistance to race 7 (fig. 3, *B*, *b*).

Reaction to Race 15

This race was lost and no tests were made with it.

Reaction to Race 9

Extensive tests of F_3 lines were made with race 9. All seedling plants regardless of genotype were susceptible, but to varying degrees, as shown in table 7. The J. W. S. parent (fig. 3, *D*, *e*) and line 575, L^2L^2rr, were susceptible. Line 584, L^2L^2RR (fig. 3, *D*, *c*), was distinctly less susceptible than line 575. Line 8, L^1L^1rr (fig. 3, *D*, *a*), appeared to be slightly less susceptible than line 584; and line 7, L^1L^1RR, and the Buda parent were classed as moderately susceptible (fig. 3, *D*, *b*, *d*). Plants of genotype L^2L^2RR and L^1L^1RR developed a type of mature-plant resistance in which relatively few pustules developed, even when the leaves were heavily inoculated.

Reaction to Races 16 and 21

The F_3 plants containing the factors conditioning rust reaction in all possible combinations, heterozygous as well as homozygous, were invariably susceptible to races 16 and 21 (fig. 3, *E*). Plants having the factors L^2L^2rr, conditioning rust reaction of the J. W. S. parent (fig. 3, *E*, *e*), were somewhat more susceptible than heterozygous ones or those having the L^1 and *R* factors (fig. 3, *E*, *a*, *b*, *c*, *d*), as indicated by pustule vigor, ability to produce an abundance of spores for a prolonged period, and compatibility between host and parasite.

Reaction of Heterozygous Plants

Under favorable conditions for growth of flax and for rust development, the effect of host heterozygosity on development of rust by races producing intermediate infection types was evident. Immunity from rust was dominant, and plants heterozygous for the immune factors L^1L^2 were immune from all races from which either parent was immune (fig. 1, *A, d; B, d*). The incomplete dominance of the resistance factor *R* was apparent in those lines homozygous for the factors conditioning immunity from either race 4 or race 7 and heterozygous for the factors conditioning semiresistance to race 3 (fig. 1, *A, b;* fig. 1, *B, b*; fig. 2, *D, b;* fig. 2, *E, b*). For example, F_3 line 431, L^1L^1Rr, in one test had 7 plants susceptible, 15 moderately susceptible, and 7 semiresistant to race 3 (fig. 1, *A, a, b, c*). Line 463, L^2L^2Rr, had 5 plants susceptible, 19 semiresistant, and 8 resistant to race 7 (fig. 1, *B, a, b, c*). These resistant plants of line 463 were grown to maturity, and, as the F_4 progeny was homozygous for resistant reaction to race 7, it was evident that differences in degree of resistance of the F_3 plants had had a genetic basis. However, it was only during periods exceptionally favorable for growth of the plant and for rust development that variations of the intermediate infection types could be considered as reliable indicators of genotypic differences.

DISCUSSION

The data presented serve to emphasize that in developing a program for breeding rust-immune varieties of flax it is important to know the inheritance of certain plant characters and to understand the interaction of the genes that condition rust reaction in the parent varieties as well as the relation between these factors and the physiologic races of the flax rust fungus.

Previous investigations had indicated that the inheritance of immunity from flax rust was conditioned by one or two dominant factors (*9, 10*). In the present investigations, however, it was found that, while immunity from specific races of rust was conditioned by dominant genes, the dominants carried by Buda and J. W. S. and controlling their immunity and susceptibility to two physiologic races are allelic. Consequently, no segregates could be obtained that were homozygous for both of the dominant allels conditioning immunity from the two races of rust. Likewise, no segregates were obtainable that were susceptible to both of the races.

Obviously, in a region where both races 4 and 7 occur, it would be futile to attempt to obtain a rust-immune variety of flax by crossing Buda and J. W. S., although each variety is immune from one of the

races and neither variety is susceptible to both. On the other hand, the existence of the allelic series of immunity factors simplifies the problem of breeding for immunity from certain physiologic races of the flax rust fungus. Thus, Ottawa 770B has been immune from all North American and European rust collections (*4*, *12*) and J. W. S. has been immune in South America (*14*). In a cross between these varieties the factors *LL* of Ottawa 770B condition immunity from North American and European races and susceptibility to a South American race (race 22), while the allelic factors L^2L^2 of J. W. S. condition immunity from South American races and susceptibility to certain North American and European races. Since susceptibility to rust is recessive, F_2 plants susceptible to race 22 are homozygous for the factors *LL* conditioning immunity from North American and European races of rust, and F_2 plants susceptible to any one of races 7, 9, 13, 15, 16, or 21 are homozygous for the factors L^2L^2 conditioning immunity from South American races. In either case, three-fourths of the plants could be discarded in the F_2 generation and subsequent tests could be confined to material homozygous for the pair of factors conditioning immunity from the races of rust with which the investigator is concerned.

The use of a line having the genotype L^1L^1rr, in addition to the regular series of differential varieties, is helpful in the identification of races of *Melampsora lini* differentiated by the semiresistant reaction of Buda. To these races Buda may be somewhat resistant in tests conducted under unfavorable conditions. Plants of genotype L^1L^1rr are susceptible, even under adverse growing conditions, to races to which Buda is semiresistant.

The reaction of Buda to the different races of the flax rust fungus, varying by almost imperceptible stages from immunity (no evidence of the formation of uredia) to susceptibility, is suggestive of the involvement of multiple factors either in the host or in the pathogen. Since no evidence was obtained that more than two factors are concerned in conditioning rust reaction in Buda, these studies suggest that the variable reaction of this variety is due to the interaction of quantitative factors inherent in the rust fungus.

SUMMARY

The inheritance of factors conditioning reaction to the flax rust fungus was studied in a cross between Buda and J. W. S., two varieties possessing differential reactions to physiologic races of *Melampsora lini*. J. W. S. is immune from 18 of the 24 races that have been identified from North and South American collections of the flax rust fungus and susceptible to races 7, 9, 13, 15, 16, and 21. Buda is immune from races 7 and 20; resistant to races 1, 5, 6, 10, and 11; semiresistant to races 3, 14, 15, 17, 18, and 23; moderately susceptible to races 2, 9, 12, 13, and 24; and susceptible to races 4, 8, 16, 19, 21, and 22.

The F_1 generation was immune from all races from which either parent was immune, indicating that immunity was dominant. Susceptible genotypes could be identified in the F_2 generation, but those having intermediate infection types required further tests of F_3 behavior.

Each F_3 line that was attacked by race 7 was homozygous for immunity from races 3 and 4. Conversely, each F_3 line attacked by race 3 or 4 was homozygous for immunity from race 7. F_3 lines homozygous for susceptibility to race 4 approximated a ratio of 1 semiresistant to 2 heterozygous to 1 susceptible to race 3. Among the F_3 lines immune from race 4, there were approximately 1 resistant to 2 heterozygous to 1 susceptible to race 7. Lines having all plants either immune from or semiresistant to race 3 had all plants either immune from or resistant to race 7. Lines segregating for immunity, semiresistance, and susceptibility to race 3 segregated for immunity, resistance, and susceptibility to race 7. Lines having all plants either immune from or susceptible to race 3 had all plants either immune from or susceptible to race 7.

The results obtained were explained by assuming that immunity from races 7 and 20 in Buda was conditioned by a pair of dominant factors allelic to the pair of dominant factors conditioning immunity from 18 races in J. W. S. The reaction of Buda and the segregation of the F_3 population suggested that Buda carried, in addition to the factors conditioning immunity from race 7, factors conditioning semiresistance to race 3 and resistance to race 7 that were independent of and hypostatic to the immune factors. On this basis the genotype of Buda would be L^1L^1RR and that of J. W. S. L^2L^2rr.

The reaction of Buda to races involved in these tests extended by almost imperceptible steps from immunity to susceptibility. Both the L^1 and the R factors appeared to be operative in conditioning resistance to races to which Buda was resistant. In tests with races to which Buda was semiresistant, moderately susceptible, or susceptible, the factors L^1L^1 had little effect, and reductions in degree of susceptibility appeared to be largely attributable to the R factor. Neither the L^1L^1 nor the RR factors had any appreciable effect on the seedling reaction to races 4, 8, 19, and 22 to which Buda is susceptible.

J. W. S., L^2L^2rr, is susceptible to races 7 and 9, but F_3 plants of genotype L^2L^2RR were resistant to race 7, and their reaction to race 9 varied from moderately susceptible to susceptible.

All plants tested were susceptible to races 16 and 21, to which both Buda and J. W. S. were susceptible.

The R factor was incompletely dominant and, under favorable growing conditions, heterozygous plants of the genotypes L^1L^1Rr or L^2L^2Rr were distinctly less resistant to races producing intermediate infection types, such as races 3 and 7, than homozygous plants L^1L^1RR or L^2L^2RR.

A line having the genotype L^1L^1rr, previously unknown, facilitates the identification of physiologic races differentiated by the semiresistant reaction of Buda.

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A GENETIC STUDY OF COMMON BEAN MOSAIC UNDER CONDITIONS OF NATURAL FIELD TRANSMISSION [1]

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INTRODUCTION

Common bean mosaic (bean virus 1) apparently occurs wherever snap beans (*Phaseolus vulgaris* L.) are grown, but in the southern part of the United States the damage is especially severe. Susceptible varieties may give complete crop failures, and tolerant varieties may develop more pronounced symptoms than they do in northern and western bean-growing areas. Because of the latter fact, it is possible in South Carolina to study the inheritance of reaction to the virus in resistant×tolerant crosses with less difficulty in identifying phenotypes than might be experienced in certain other climatic areas.

In order to obtain a high percentage of virus transmission, most workers with common bean mosaic have employed artificial inoculations. In field plots of the United States Regional Vegetable Breeding Laboratory, Charleston, S. C., natural field spread of common bean mosaic has produced the maximum possible transmission; and, with suitable controls for insuring the identity of the virus, it is possible to rely on field data with a high degree of confidence. The data reported herein were obtained incidentally in a study of bacterial blight resistance in snap beans. The data are submitted because they lead to a different interpretation of the genetics of common bean mosaic from any hitherto recorded.

MATERIALS AND METHODS

The crosses observed in detail were Stringless Black Valentine× U. S. No. 5 Refugee and the reciprocal. U. S. No. 5 Refugee is highly resistant to common bean mosaic [2] as reported from various places. During the last 5 years several thousand plants of this variety have been observed at Charleston, S. C., and no common bean mosaic (bean virus 1) or other virus diseases have been found except in the case of a few obvious rogues. Stringless Black Valentine under early-spring conditions at Charleston, and under normal conditions in the seed-growing areas of the northern and western parts of the United States, ordinarily gives a tolerant reaction to infection with common bean mosaic; i. e., the disease may be present, but the injury is slight or undetectable. Plantings of Stringless Black Valentine made only a few weeks later at Charleston, however, may show much more pronounced symptoms and injury. Other tolerant varieties like Bountiful and U. S. No. 1 Refugee respond much like Stringless Black Valentine.

[1] Received for publication May 7, 1941. This work was performed chiefly under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] WADE, B. L., and ZAUMEYER, W. J. U. S. NO. 5 REFUGEE, A NEW MOSAIC-RESISTANT REFUGEE BEAN. U. S. Dept. Agr. Cir. 500, 12 pp., illus. 1938.

Checks for each generation included the two parents (Stringless Black Valentine and U. S. No. 5 Refugee) and in addition, for the F_1 and F_2 generations, samples of inoculated and uninoculated Stringless Green Refugee. Since Stringless Green Refugee is very susceptible to common bean mosaic, and since mosaic is to some extent a seed-borne disease, a mosaic-free strain was grown under cheesecloth for testing the virus present in Stringless Black Valentine and in the susceptible F_2 segregates. Inoculation of the Stringless Green Refugee in these two cases for the purpose of verifying the presence of the common bean mosaic virus were the only artificial inoculations made.

The F_1 and F_2 plants were grown in rows adjacent to the parents; but in the F_3 generation, as all border rows were U. S. No. 5 Refugee and Stringless Black Valentine, readings were made on the border rows for checks. The borders were about 24 rows apart.

The first observations on common bean mosaic at the Vegetable Breeding Laboratory were made in the spring of 1936. Varieties commonly classified as tolerant showed severe symptoms, and susceptible varieties such as Stringless Green Refugee were so badly injured that only a few seeds per plant were produced. Of 453 U. S. No. 5 Refugee plants observed, none showed any symptoms of mosaic, while in all other varieties noted all plants were infected. The varieties and the number of plants observed were as follows: Stringless Black Valentine, 338; Bountiful, 507; Stringless Green Refugee, 554; and Thousand to One Refugee, 321.

In order to verify the presence and identity of the common bean mosaic virus, seed was harvested from the above-mentioned plants and two series were planted in the greenhouse in the spring of 1937. Sufficient seed was planted for at least 100 plants in each series, and readings were taken on primary or seed-borne mosaic on the first 100 in each case. Except in the case of U. S. No. 5 Refugee, western-grown seed was used as a check. The results from seed from eastern infected plants and from western seed, respectively, were as follows: Stringless Black Valentine from eastern plants infected with seed-borne mosaic, 9.5 percent, and from western seed, 3 percent; Bountiful, 4 percent and 2 percent; Stringless Green Refugee, 25.5 percent and 8.5 percent; Thousand to One Refugee, 17 percent and 29.5 percent; U. S. No. 5 Refugee, 0 percent. From 5 Stringless Black Valentine plants of Charleston origin and 2 of western origin showing primary symptoms, inoculations were made into 10 plants of healthy Stringless Green Refugee. Bountiful and Stringless Green Refugee were also inoculated by using 2 primary infections from the Charleston source and 2 of western origin. In all cases typical symptoms of common bean mosaic were observed. Of 70 Stringless Green Refugee plants inoculated from primary-infected Stringless Black Valentine, 54 showed symptoms, while 10 check plants inoculated from healthy Stringless Black Valentine remained mosaic-free. Of 70 plants of Bountiful, 57 became infected and the 10 checks remained healthy; of 40 plants of Stringless Green Refugee, 32 became infected and the 10 checks remained healthy. The symptoms in all cases were characteristic of Common bean mosaic.

As aphids are usually assumed to be responsible for a large part of the secondary spread of bean mosaic in the field, records have been kept on the aphid population for the last 5 years with readings on

approximately May 1, 10, and 20. No attempts have been made to identify the various species found. Ten plants from each of 10 rows taken at random have furnished the basis for the average in each case. The average number of aphids present by May 1 was approximately 1 per plant, whereas by May 10 the number had increased to 2.5, and by May 20 had decreased to about 1.5. Aphids are not ordinarily found in abundance on beans, but it is believed that the numbers found could be responsible for a considerable epidemic of common bean mosaic.

RESULTS

The F_1 generation grown in the spring of 1938 consisted of 9 plants, 3 from the cross Stringless Black Valentine × U. S. No. 5 Refugee and 6 from the reciprocal. These plants all remained free from virus diseases, as did the 37 plants of the U. S. No. 5 Refugee parent; but all 42 of the Stringless Black Valentine parent plants showed symptoms of common bean mosaic. Inoculations made from the Stringless Black Valentine parent to healthy Stringless Green Refugee indicated that common bean mosaic virus alone was present in the Stringless Black Valentine plants (table 1).

In the F_2 generation a ratio of 3 resistant to 1 tolerant was obtained with a total of 179 plants observed, and there was no significant difference in the reciprocals. Chi-square tests indicated satisfactory conformation to a 3:1 ratio. Again the resistant parent remained healthy, while all 106 Stringless Black Valentine plants showed symptoms. Inoculations made from the susceptible F_2 segregates to Stringless Green Refugee showed that the virus responsible for the disease was the common bean mosaic virus (table 1).

TABLE 1.— *Results obtained with F_1, F_2, and F_3 progenies from the crosses Stringless Black Valentine × U. S. No. 5 Refugee (A) and reciprocal (B)*

Cross	Classification of plants in F_1 generation		F_2 generation			F_3 generation								
			Classification of plants			Classification of families				Classification of plants in F_3 segregating families		Chi square for 3:1 ratio for plants in F_3 segregating families		
	Resistant	Tolerant	Resistant	Tolerant	Chi square[1] for 3:1 ratio	Resistant	Segregating	Tolerant	Chi square for 1:2:1 ratio in F_3 families[2]	Resistant	Tolerant	Accumulated[3]	Population[1]	Heterogeneity[4]
	No.	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*		*No.*	*No.*			
A	3	0	77	23	0.213	6	15	4	1.340	404	108	13.329	4.167*	9.162
B	6	0	60	19	.038	6	16	5	1.018	496	140	19.746	3.027	16.719
Total	9	0	137	42	.225	12	31	9	2.270	900	248	33.075	7.066**	26.009

[1] 1 degree of freedom.
[2] 2 degrees of freedom.
[3] Chi square for individual progenies added, with 1 degree of freedom for each family.
[4] Obtained by subtracting population chi square from accumulated chi square. Degrees of freedom, 1 less than number of families.

In the spring of 1939, at the time the F_2 plants previously discussed were grown, some F_3 families from an F_2 plant grown in the fall of 1938 were observed to be segregating in a ratio of 1 resistant to 2 segregating to 1 tolerant. Because of the presence of a considerable amount of bacterial blight an exact count of plants was not made, but in the cross Stringless Black Valentine × U. S. No. 5 Refugee the family ratio was 12 resistant to 23 segregating to 6 tolerant; that of the reciprocal, 3:6:4. None of the chi-square values approached significant deviations, and again there was no indication of differences between reciprocals. Since these observations were preliminary, they are not included in table 1.

The F_3 generation grown in the spring of 1940 consisted of 25 families from the cross and 27 from the reciprocal. The combined data showed 12 resistant, 31 segregating, and 9 tolerant families with a nonsignificant chi-square value. Again it was indicated that there was no significant difference in genetic behavior of the cross and its reciprocal.

The F_3 plant population from the 31 segregating families showed a highly significant deficiency of recessives, but heterogeneity chi square showed no difference in the cross and its reciprocal. The F_3 results are shown in detail in table 1. All 104 U. S. No. 5 Refugee check plants were healthy, whereas all 93 Stringless Black Valentine check plants were infected with common bean mosaic virus.

DISCUSSION

In 1921 McRostie [3] reported crosses with various field beans, including Michigan Robust, in which he found resistance to be at least partially recessive. By grouping his data he obtained an F_2 ratio approaching 9 susceptible to 7 resistant. Part of his work was carried out by artificial inoculations, but most of it depended on field spread from susceptible, primarily infected plants.

In 1935 Pierce [4] reported results with various crosses. Robust crossed with Stringless Green Refugee gave a mosaic-susceptible F_1 generation, but Robust crossed with a strain of Great Northern or with Corbett Refugee gave resistant F_1 plants in both cases. Corbett Refugee × Stringless Green Refugee and the reciprocal were resistant in the F_1 generation, and in the F_2 generation they gave 88.8 percent and 82.2 percent resistant, respectively. Idaho Refugee and Wisconsin Refugee behaved in crosses with Stringless Green Refugee about like Corbett Refugee. Pierce did not attempt a genetic explanation of his results.

In 1936 Parker [5] reported differential results from crosses of Stringless Green Refugee × Michigan Robust and the reciprocal, as well as with Corbett Refugee × Stringless Green Refugee. Parker interpreted differences in reciprocals in part on the basis of cytoplasmic inheritance. When Stringless Green Refugee was used as the female parent, some of the results approached a 3:1 ratio of resistant to susceptible.

[3] McRostie, G. P. Inheritance of disease resistance in the common bean. Jour. Amer. Soc. Agron. 13: 15–32. 1921.

[4] Pierce, W. H. The inheritance of resistance to common bean mosaic in field and garden beans. Phytopathology 25: 875–883, illus. 1935.

[5] Parker, M. C. Inheritance of resistance to the common mosaic virus in the bean. Jour. Agr Res. 52: 895–915, illus. 1936.

In the three genetic studies mentioned the contrasts involved were crosses between resistant and susceptible strains, whereas in the present paper the work is limited to resistant with tolerant. Groupings of resistant, tolerant, and susceptible varieties have been used by many investigators; as, for example, Clark.[6]

Pierce [7] was careful in his work to exclude the possibility of contamination with yellow bean virus (bean virus 2), and both Parker [8] and Pierce used artificial inoculation instead of depending upon field spread. In the work reported herein reliance was placed upon field spread and no artificial inoculations were made except as a check upon the technique and to test for the presence of the common bean mosaic virus. Extensive observations on U. S. No. 5 Refugee, which is susceptible to yellow bean virus, and on other varieties, as well as considerations of the seed-borne nature of the disease observed, indicate that yellow bean virus could not have been present to complicate the results reported herein.

Johnson [9] recently interpreted some symptoms observed on beans from inoculations from apparently healthy *Lathyrus tingitanus* as being due to an allergic response of the bean plants. It seems possible, if allergic responses do occur, that there might be allergic responses of one strain of beans to another and that they might be much more severe with artificial inoculations than with natural insect transmission; consequently artificial inoculations might per se introduce a factor rather disturbing to genetic ratios.

It seems possible that part of the irregularity of ratios encountered by both Pierce and Parker may have come from the presence of a partially lethal variegation in Corbett Refugee and the two strains derived from it, Idaho Refugee and Wisconsin Refugee. U. S. No. 5 Refugee, although related to Corbett Refugee, does not carry the factor or factors for variegation.

SUMMARY AND CONCLUSIONS

In a cross of Stringless Black Valentine × U. S. No. 5 Refugee and the reciprocal, the results with the F_1, F_2, and F_3 generations indicated that the resistance of U. S. No. 5 Refugee to common bean mosaic virus is dominant to the tolerance (which showed a definitely mosaic pattern with marked mosaic symptoms at Charleston, S. C.) of the Stringless Black Valentine, and that a single factor is responsible for the resistance.

There were no significant differences between the cross and its reciprocal.

There was a slight but cumulative deficiency of recessives (22 percent of the population tolerant, theoretical 25 percent), which reached significance in the total populations from segregating families in F_3.

[6] CLARK, N., compiler. MOSAIC RESISTANT CANNING BEAN SHOWS PROMISE. Wis. Agr. Exp. Sta. Bul. 421: 60-62, illus. 1931.

[7] See footnote 4.

[8] See footnote 5.

[9] JOHNSON, JAMES. INOCULATION OF BEAN WITH EXTRACTS FROM OTHER HEALTHY LEGUME SPECIES. (Abstract) Phytopathology 30: 12. 1940.

GROWTH SUBSTANCE IN CROWN GALL AS RELATED TO TIME AFTER INOCULATION, CRITICAL TEMPERATURE, AND DIFFUSION [1]

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INTRODUCTION

The importance of growth substances in the normal development of plant tissues has suggested that, when present in unusual amounts, they may be more or less directly responsible for atypical and pathological growth. Attention has been directed especially to such growths as crown gall caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. Beta-indole-acetic acid and substances related in effect, like auxin-a and auxin-b in particular, have been considered the chemical means by which the bacteria induced this diseased overgrowth.

The evidence for this has been presented and discussed by various workers. For example, beta-indole-acetic acid has been suggested with various degrees of emphasis by Němec (*11*), Brown, and Gardner (*2*), Link, Wilcox, and Link (*5*), and Berthelot and Amoureux (*1*) as the responsible stimulating agent produced by the bacteria. Locke, Riker, and Duggar (*6*) described symptoms on plants bearing crown gall as similar in several particulars to those induced by beta-indole-acetic acid. Limited trials by diffusion technique indicated that more growth substance is present in recently inoculated tomato stems than in control stems. However, trials with older galls and with extractions failed to show differences between gall and control tissue. In addition, Locke, Riker, and Duggar (*7*, *8*) found that crown gall and related avirulent bacteria in peptone broth produced in similar amounts a substance acting like beta-indole-acetic acid in its sensitivity to acid and alkali, and that both healthy and diseased tomato tissue contained material likewise similar to this acid. Kraus, Brown, and Hamner (*4*) demonstrated the similarity in structure between galls induced by *Phytomonas tumefaciens* and beta-indole-acetic acid.

The present investigations have been aimed at quantitative determinations of certain growth substances (1) that are found at progressive intervals following crown gall inoculation, (2) that are present in tomato stems grown both above and below the critical maximum temperature for crown gall formation, and (3) that diffuse from healthy and diseased tissue. A preliminary statement of some of this work has already appeared (*13*). With such studies it seemed possible to clarify the relation, if any, of growth substances such as beta-indole-acetic acid to the pathogenicity of crown gall bacteria.

[1] Received for publication, December 21, 1940. This work was supported in part by the International Cancer Research Foundation, and assistance in making tests was furnished by the personnel of the Work Projects Administration, Official Project No. 65-1-53-2349. Dr. C. Eisenhart, station statistician, kindly interpreted the analysis of variance.

[2] Italic numbers in parenthesis refer to Literature Cited, p. 405.

MATERIALS AND METHODS

The tomato (*Lycopersicum esculentum* Mill.) was used as a convenient host. Plants were grown individually in 4-inch pots in the greenhouse at approximately 24° C. and inoculated by stem punctures when they were about 8 inches tall. Galls were conspicuous in about 10 days and equal to the diameter of the stem in 3 to 4 weeks. Suitable control punctures were always made in a separate series of similar plants.

The growth substance was extracted by a slight modification of the method of Van Overbeek (*16*). A 25-gm. sample of fresh plant tissue was cut into pieces about 2 mm. in thickness and placed in a 500-ml. Erlenmeyer flask. About 250 ml. of freshly distilled peroxide-free ether was added immediately and the whole placed overnight at 4° C. The following morning the ether was separated from the plant material and the water. The extract-containing ether was heated in a distillation apparatus on a steam table until only about 2 ml. remained. The distilling flask was cooled under a cold water tap and the ether poured into a 10-ml. vial. One ml. of distilled water was pipetted into the vial, which was then placed on the steam table until the remaining ether had evaporated. Thus the ether extract of 25 gm. of plant tissue was contained in 1 ml. of water. This 1 ml. of water was mixed with an equal volume of 3-percent agar, and the mixture was tested for the presence of growth substance. Tests were always made on the same day that the extracts were prepared. The galls and control tissue were treated identically in extraction. While it is recognized that the ether extraction may not remove all of the free growth substance present, it appears that the relative amounts obtained from the control tissue and the crown gall tissue should be comparable.

Considerably higher yields of growth substance have been secured with other methods from plant tissues by various workers, including Avery, Berger, and Shalucha.[3] Apparently these higher yields are due largely to hydrolysis of precursors rather than to improved extraction of free auxin. The method used seems one of the best at present available for securing free auxin.

The standard *Avena* test (*18*) was used, with slight modifications as explained by Locke, Riker, and Duggar (*7*), in determining the amounts of growth substances present. It is apparent that the growth substances measured were those acting upon *Avena* coleoptiles under the conditions of experimentation. Obviously, various growth substances may have been present which were not detected by this technique.

The measurements secured are based upon averages of curvatures of 24 to 36 coleoptiles. Thus, the data included in this paper, together with those from various preliminary trials not mentioned because of the space they would occupy, are based upon about 7,000 coleoptile measurements.

The critical-temperature studies were made in two cubical chambers 4 feet square, each illuminated by ten 30-inch 30-watt daylight fluorescent lamps. The plants received light for 12 to 15 hours each day. The temperature was thermostatically maintained at 27° C. in

[3] AVERY, GEORGE S., BERGER, JULIUS, and SHALUCHA, BARBARA. THE TOTAL EXTRACTION OF FREE AUXIN AND AUXIN PRECURSOR FROM PLANT TISSUE. Amer. Jour. Bot. 28:596-607, illus., 1941.

one chamber, where galls were formed, and at 31° in the other, where no galls developed. This is below and above, respectively, the maximum temperature for gall formation determined by Riker (*12*) and confirmed by the present studies.

EXPERIMENTAL RESULTS

COMPARISON OF RESULTS IN TERMS OF COLEOPTILE CURVATURE AND OF HETEROAUXIN EQUIVALENTS

The results of the *Avena* test are commonly reported in the average amount of curvature produced. This seems subject in certain localities to the error of variations from day to day in sensitivity of the test plants. Some variations also occur within a given day. In these studies results were taken on known material several times within a day, as well as on different days, and a comparison was made of the variations secured. The results showing "within-a-day" and "day-to-day" variations are given in table 1, and an analysis of their variance (*15*) in table 2.

The variation between days has an F value of 10.96 that greatly exceeds the appropriate 5-percent point (2.15); so the existence of a day-to-day variation can be inferred. Consequently, the data presented later are corrected to heteroauxin equivalents in the interest of accuracy.

TABLE 1.—*Variation in Avena coleoptile curvature, with a concentration of 100 gammas per liter of beta-indole-acetic acid, compared within the same day and on different days, 1940*

Date	Average negative curvatures for replication—			Totals
	1	2	3	
	Degrees	*Degrees*	*Degrees*	*Degrees*
Feb. 20	11	12	11	34
Mar. 3	9	7	9	25
Mar. 5	11	8	7	26
Mar. 10	9	8	7	24
Mar. 26	10	7	12	29
Mar. 27	8	8	7	23
Mar. 31	15	14	13	42
Apr. 7	11	10	12	33
Apr. 10	14	15	16	45
Apr. 14	8	6	8	22
Apr. 28	8	7	7	22
May 12	10	7	9	26
May 19	11	9	10	30
Total	135	118	128	381

TABLE 2.—*Analysis of variance on data from table 1*

Source of variance	Degrees of freedom	Sums of squares	Mean square	F	F at the 5-percent point
Between days	12	219.59	18.30	10.96	2.15
Within days	26	43.33	1.67		
Total	38	262.92			

PROCEDURE WITH ANALYSIS OF VARIANCE

The statistical procedure employed with the analysis of variance in table 2 is briefly given. Denoting by σ^2 the variance of determinations made on a single day, and by σ_d^2 the variance due to day-to-day variations (the actual days involved being regarded as a random sample from a "population" of days), one can estimate these quantities as follows:

$$\text{Est. } \sigma^2 = \text{Within days mean square} = 1.67$$

$$\text{Est. } \sigma_d^2 = \frac{(\text{Between days mean square}) - (\text{Within days mean square})}{3} = 5.54$$

where the divisor 3 represents the three determinations made each day.

The variance of the mean, $\bar{x}$, of the three determinations on a random day is

$$\sigma_{\bar{x}}^2 = \sigma_d^2 + \frac{\sigma^2}{3}$$

where σ_d^2 represents the variation arising from drawing a random day and $\frac{\sigma^2}{3}$ represents the inaccuracy arising from the fact that sensitivity for that day is estimated by only three determinations. In the day-to-day variation, σ_d^2 represents the variation which would still be present if an infinite number of determinations were made each day—that is, if the time value for the day were determined exactly—so $\sigma_{\bar{x}}^2$ will always exceed σ_d^2 no matter how accurately one determines the sensitivity prevailing on particular days. The "error" in determining the value for a particular day is always of the form $\frac{\sigma^2}{n}$ and can be reduced by increasing n (the number of replicates).

Substitution of the estimated values of σ^2 and σ_d^2 yields $\sigma_{\bar{x}}^2 = 5.54 + 0.56$; so day-to-day variation constitutes about 90 percent of the error in a mean of three determinations made on a single day.

A correction for the day-to-day variation secured seemed desirable.

CALCULATION OF HETEROAUXIN EQUIVALENTS

A correction based on the activity of a known substance is called for because many factors, beyond the practical control of the investigators, may influence the sensitivity of the coleoptiles from day to day and from laboratory to laboratory, but their consideration is beyond the scope of this paper. This correction necessitated the use of a known concentration (100 gammas per liter) of beta-indole-acetic acid with each assay of the unknown materials. The formula used in making conversions to the heteroauxin equivalents, shown at the right of succeeding tables, was adapted from that of Van Overbeek (*16*) It is as follows:

$$\frac{C \times I \times V}{W} = \text{gamma heteroauxin equivalents per liter of water in the tissue}$$

where C is the coleoptile curvature, in degrees, produced by the plant extract; I is the concentration of heteroauxin, in gammas per liter, required to give a curvature of 1 degree; V is the volume in milliliters of agar and water in which the extract was taken up; and W is the water content, in grams of plant tissue extracted.

COMPARISON OF INOCULATED AND CONTROL TISSUE AT SUCCESSIVE TIME INTERVALS AFTER INOCULATION

Measurements of growth substances have been made to clarify the suggestions about their importance in gall formation. Half of a series of tomato plants were puncture-inoculated with crown gall bacteria, and the others were given control punctures. It was considered that if auxin (i. e., any of the growth substances causing negative *Avena* curvature) was responsible for crown gall development, differences in auxin content might appear between gall and control tissue. Since the auxin content might fluctuate in relation to the progress of gall development, examinations were made at about 1, 4, 8, and 16 days after inoculation. On the last day proliferation was well under way.

The auxin in the growing tip was removed from the complex in three series by using only decapitated plants. In three other parallel series whole plants were used. The work was done in the early part of 1939.

As representative of the work done, detailed results of the series inoculated February 18 are given in the upper part of table 3.

TABLE 3.—*Crown gall and control tissue compared at progressive intervals in regard to amounts of growth substances that produce Avena coleoptile curvatures and their heteroauxin equivalents, 1939*

Plant series	Time after treatment	Average negative curvature [1]			Heteroauxin equivalent [1]	
		Inoculation	Puncture control	Heteroauxin control [2]	Inoculation	Puncture control
	Days	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*
Decapitated plants: Results in series inoculated Feb. 18	1	5	4	9	5	4
	4	12	12	11	10	10
	8	7	6	11	6	5
	16	2	3	9	2	3
Averages of results in 3 series inoculated Feb. 18, Feb. 22, and Mar. 8	1	6	5	9	6	5
	4	9	8	12	7	6
	8–9	6	6	10	5	5
	16–17	6	5	9	5	5
Whole plants: Averages of results in 3 series inoculated Apr. 5, Apr. 19, and May 3	1	9	10	8	10	10
	4	12	11	10	11	10
	8	10	8	8	12	9
	15–18	10	10	9	10	10
Averages of all trials	1	7.5	7.2	8.7	7.7	7.4
	4	10.8	9.8	11.0	8.8	7.9
	8–9	8.1	7.3	9.2	7.8	7.0
	15–18	8.4	8.1	9.2	8.1	7.8
Averages of all trials corrected on basis of total nitrogen content	1				7.0	7.4
	4				8.0	7.9
	8–9				7.1	7.0
	15–18				7.4	7.8

[1] Expressed in nearest whole number except averages of all trials.
[2] A concentration of 100 gammas per liter was used.

An example of the conversion to heteroauxin equivalents is given with the first calculation in table 3, where 5 degrees of curvature are equivalent to 5 heteroauxin equivalents. The degrees curvature are given in the table in terms of the nearest whole number, namely, 5, although the actual figure in this case was 4.7, this being the average curvature in degrees of 24 coleoptiles. Thus, C is 4.7; I is 100 gammas per liter divided by 9 degrees of curvature or 11.1; and V is 1 ml. of agar plus 1 ml. of material, or 2. Since greenhouse galls contain about

10.3 percent of dry matter (*10*) in 25 gm. of tissue, there are 2.6 gm. of dry matter and 22.4 gm. of water; thus W is 22.4. (Since there seems to be no significant difference between the percentage of dry matter in greenhouse galls and control stems, the same correction factor is used here for both gall and control tissue.) In this case the heteroauxin equivalents are about 4.6 gammas per liter of water in the plant tissue. The nearest whole number is 5, which appears in table 3. Detailed results of other trials are omitted because of their volume.

The results with decapitated plants in the series inoculated February 18, February 22, and March 8, respectively, are summarized in table 3. For making these averages the daily determinations to one decimal point were used. There is an average variation from 5 to 7 gammas of heteroauxin equivalents per liter of liquid in gall tissue and from 5 to 6 in that of puncture controls.

Whole plants were used in three series, also summarized in table 3. Except for higher auxin values throughout, the results are similar to those with the decapitated plants. Since the growing tip is a ready source of auxin, the greater amount found was anticipated.

The galls developed well in decapitated plants in which only half as much of auxin was detected as in whole plants. This in itself indicates that gall development is independent of auxin change from 5 to 10 gammas per liter.

The summary of all the tests is given in table 3. The greater amount of growth substance on the fourth day after inoculation does not seem significant. It appears that there is a slight increase in the amount of growth substance in the inoculated tissue at each average time interval over that in the control puncture. When one recalls that 8 gammas per liter represents 8 parts in 1,000,000,000, one recognizes that the difference between 8.8 and 7.9 is hardly significant. Even this difference disappears when a suitable correction is added.

The living protoplasm is reasonably considered to be the portion of the tissue active in the production of growth substances. So a comparison of gall and control tissue on a total nitrogen basis may be a closer approach to an estimation of the amount of living substance than is a comparison on a weight basis. Nagy et al. (*10*) showed that in greenhouse galls total nitrogen was 3.3 percent of the dry matter, while in corresponding stems it was 3.0 percent. A comparison of auxin values on a total nitrogen basis is doubtfully applicable where plants have been recently inoculated since there is then only a slight development of gall tissue. However, when such corrections are applied to the averages of all these trials, the results are as shown at the bottom of table 3. Here any indication of a significant difference between gall tissue and control tissue seems to disappear.

COMPARISON OF TISSUES GROWN ABOVE AND BELOW CRITICAL TEMPERATURES FOR GALL FORMATION

The auxin content of tomato tissue grown at 27° C., where galls develop, and at 31°, where no galls develop (*12*), was studied early in 1940. Experimental plants were puncture-inoculated at 1- to 2-inch intervals in several internodes. They were grown in two cubical chambers about 4 feet on a side located in the basement of the greenhouse. With rare exceptions there was a variation from the set temperatures (27° and 31°) of less than 1°. The plants in each chamber were illuminated from above for 12 to 15 hours by 10 daylight

fluorescent, 30-inch, 30-watt lamps with aluminum reflectors about 30 inches above the pots or boxes. No effort was made to regulate the humidity of the air, which fluctuated in both chambers around 65 percent of saturation. Fresh air was supplied by forced ventilation. The plants were harvested 4 to 5 weeks after treatment.

Fifteen-gram samples of gall tissue, and of stem tissue from between the galls, were taken from plants grown at 27° C. A similar sample of stem tissue was taken from between the inoculation points on plants grown at 31°. The tissues were ether-extracted as described earlier and the extracts assayed on *Avena* coleoptiles.

The results from temperature studies are shown in table 4, which is arranged in a manner similar to table 3. In general, the amount of growth substance found was similar to that in whole plants, as recorded in table 3. The difference in the amounts of growth substances secured from the stems at 27° and 31° C. is not considered significant as far as gall formation is concerned. It is much less than that between the whole and decapitated plants (table 3), and even in the latter instance gall development was not significantly influenced. Whatever difference exists in the temperature series can probably be attributed to less vigorous growth at 31° than at 27°. Corrected on a total-nitrogen basis the averages show approximately 2 gammas per liter more of growth substances in the galls than in the controls. This is not considered significant and possibly can be explained by the more active growth of the gall tissue than the adjacent stem tissue of the same plant.

TABLE 4.—*Crown gall and control tissue compared at 27° and 31° C. in regard to the amounts of growth substances that produce Avena coleoptile curvature and their heteroauxin equivalents, 1940*

Date	Average negative curvature [1]				Heteroauxin equivalents		
	Gall at 27° C.	Stem at 27° C.	Stem at 31° C.	Heteroauxin control [2]	Gall at 27° C.	Stem at 27° C.	Stem at 31° C.
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*	*Gammas per liter*
Jan. 15	23	14	13	19	19	12	10
Mar. 16	15	13	16	15	16	14	17
Apr. 2	14	12	10	13	17	14	12
Apr. 26	21	12	.....	17	20	11	.....
May 28	12	12	10	16	11	11	9
Averages of all trials	17.2	12.8	12.3	15.8	16.3	12.1	11.6
Averages corrected on total nitrogen basis	.....	.....	.....	.....	14.8	12.1	11.6

[1] Expressed in nearest whole number except for averages.
[2] A concentration of 100 gammas per liter.

Although no significant differences were found in the amount of growth substances either in crown gall and control tomato tissue or in tomato tissues grown above and below the critical temperatures, there are two remaining possibilities. More auxin might have been produced in the gall than in control tissue, but it might have disappeared rapidly because (1) it was decomposed during gall formation, or (2) it was diffused away so rapidly that it failed to accumulate. Evidence about

the first is difficult to secure. The data from decapitated and whole plants in table 3 seem not to support this possibility. The second possibility was studied by means of diffusion technique.

DIFFUSION FROM GALL AND CONTROL TISSUE

The amount of growth substance which diffuses from crown gall and control tissue was studied by a technique similar to that employed by Went (*17*) with the tips of *Avena* coleoptiles. Tomato plants, from which gall and control tissues were obtained, were grown in the greenhouse and inoculated under conditions analogous to those in the extraction experiments. The materials were tested for diffusible growth substances at 1, 4, 8, and 16 days after inoculation.

Two stem segments with a combined weight of 500 mg. and with one inoculation point each were cut out with a razor blade. In several experiments no growth substances were obtained by diffusion from crown gall tissue itself. For this reason, about 2 mm. of stem tissue was left below the inoculation point, or gall tissue, when the inoculated segments were removed from the plant. The lower end of each section was thoroughly washed with a "salt solution" composed of NaCl, 6 gm.; KCl, 0.4 gm.; $CaCl_2$, 0.4 gm.; and H_2O, 1,000 ml. (*3*). This removed the exudate from the cut cells and supplied a moisture film to facilitate diffusion. The stem segments were then placed upright on agar blocks measuring 1 by 8 by 11 mm. and consisting of 1½ percent agar in the salt solution. The blocks were on glass microscope slides. The slides with their corresponding agar blocks and stem segments were placed in Petri dishes lined with wet filter paper for 2 hours at laboratory temperature. Following this diffusion the agar blocks were each cut into 12 equal parts and applied to *Avena* coleoptiles. Similar treatment was given tissue with control punctures. The results are correspondingly based on the average curvature of 24 coleoptiles for each material per test.

The polarity of growth substance movement was apparent since no active substance was obtained in trials when stem segments were placed top down on the agar blocks.

TABLE 5.—*Crown gall and control tissue compared at progressive intervals in relation to the diffusion of growth substances, 1939*

Trial	Time after treatment	Average negative curvature [1]			Heteroauxin equivalents [1]	
		Inoculation	Puncture control	Heteroauxin control [2]	Inoculation	Puncture control
	Days	*Degrees*	*Degrees*	*Degrees*	*Gammas per liter*	*Gammas per liter*
Trial of Oct. 8	1	14	15	17	33	36
	4	9	2	12	29	8
	8	11	8	12	35	27
	16	9	10	9	39	40
Averages of 4 trials	1	9.5	10.5	12.2	30.5	33.7
	4	10.4	7.0	16.5	24.7	16.6
	8	9.9	7.5	15.1	25.7	19.5
	16	7.5	7.9	8.7	33.8	35.6
Averages corrected on total nitrogen basis	1	.....	.....	.....	27.7	33.7
	4	.....	.....	.....	22.5	16.6
	8	.....	.....	.....	23.4	19.5
	16	.....	.....	.....	30.7	35.6

[1] In nearest whole number except for averages. [2] A concentration of 100 gammas per liter.

Numerous preliminary experiments were conducted to determine the relation of coleoptile curvature to (1) the area of the diffusion surface, (2) the diffusion time, and (3) the wet weight of the sample. The results were quite variable. Within the limits of the studies no significant relation was determined between these factors and the coleoptile curvatures. The details of results are omitted. Since inoculated and control tissues were similar respecting these factors, the results given in table 5 are primarily comparative. The values, while within the supposed range of quantitative coleoptile sensitivity, contain objectionable variations. One representative trial appears in table 5. The average of 4 trials is given with corrections on the total-nitrogen basis.

As in previous trials, there was no significant difference between the amount of growth substance secured from inoculated stems and from those receiving only control punctures. Thus, it appears that the diffusion of growth substances from crown gall is not significantly higher than that from control tissue.

DISCUSSION

The mechanism by which the crown gall bacteria induce the characteristic pathological cell growth has been investigated by a number of research workers, as explained in the introduction. Recently the chemical cause of this diseased growth has been suggested or claimed to be beta-indole-acetic acid or some closely related substance elaborated by the bacteria during growth in the host tissue. This concept has been based upon several lines of evidence: (1) The crown gall bacteria produce beta-indole-acetic acid or a similar substance in suitable cultures; (2) extracts of the crown gall culture and of crown gall tissue when applied to suitable plants have induced proliferation more or less closely resembling crown gall tissue; and (3) preparations of beta-indole-acetic acid when applied to suitable tissue have also induced proliferation closely resembling crown gall.

From these lines of evidence it has appeared to some that beta-indole-acetic acid is the active bacterial metabolite and that the crown gall problem has been solved. All of these different lines have been studied and confirmed in the writers' laboratories. However, certain additional experiments have been made in parallel with these investigations. They have shed further light upon the problem and cast doubt upon the validity of certain conclusions.

The necessity for rigid controls on experiments dealing with the mechanism of cell stimulation in crown gall and some suggestions regarding them have been indicated by Riker and Berge (*14*). When such controls were applied to the present investigation, it appeared that not only the crown gall organism but various nonpathogenic bacteria produced beta-indole-acetic acid or closely related growth substances in suitable culture media. On the most favorable medium for this production the maximum amount of growth substances ever secured was about 125 gammas per liter (*7*).

A variety of extracts from plants and also various chemicals are capable of inducing proliferations in plants. Scores of such substances have been listed in the review by Riker and Berge (*14*). The overgrowths induced by beta-indole-acetic acid are histologically similar in many respects to those induced by crown gall bacteria (*4*). Although 1 percent and sometimes smaller amounts may be effective

on some plants, the common dosage is 3 percent in lanolin, which corresponds to 30,000,000 gammas per liter. When used for inducing roots in cuttings, aqueous solutions of beta-indole-acetic acid are commonly diluted to less than 1 in 20,000 (50,000 gammas per liter). The concentrations occurring, therefore, in bacterial cultures and in plant tissues are hundreds or thousands of times smaller than those employed for inducing pathological growth with chemicals.

The "host range" of beta-indole-acetic acid is somewhat different (*13*) from that of crown gall bacteria. There are some plants that respond to this substance that are not susceptible to crown gall, while there are others susceptible to crown gall that do not respond to beta-indole-acetic acid.

Thus, it appears from the evidence presented here (1) that the present writers have confirmed the experimental results (mentioned earlier) of other investigators, (2) that they have provided new evidence shedding further light upon the interpretations, and (3) that these interpretations need revision. So far as the present writers are aware, there is no satisfactory evidence showing that the pathogenicity of crown gall bacteria can be attributed to the production of heteroauxin or similar growth substances measured by the technique here employed.

However, the crown gall bacteria produce various growth substances other than auxin use, in fact, everything they require for growth in a purely synthetic medium. Measurements reported by McIntire, Riker, and Peterson (*9*) have shown the synthesis by this organism of unusually large quantities of biotin, large amounts of flavin, and moderate amounts of thiamin and pantothenic acid. This ability of the crown gall organism to manufacture other growth substances needs further study in relation to its cell-stimulating property.

SUMMARY

Growth substances of the heteroauxin group have been studied in relation to crown gall formation.

About 90 percent of the variations in the *Avena* coleoptile test as used were eliminated by taking the results in terms of heteroauxin equivalents.

No significant differences in auxin content were found between inoculated and control tissue 1, 4, 8, and 16 days after inoculation, especially when comparisons were made on a total nitrogen basis.

Galls and control stems from decapitated tomatoes contained approximately half as much auxin as those from whole plants. However, galls were similar in size on both kinds of plants.

No significant difference was found between the auxin content of tomato stems grown at 27° C., where galls develop, and at 31°, where galls do not develop.

No significant difference was found in the amount of auxin diffusing from stems bearing galls and from control stems.

The experiments of certain other investigators, from which suggestions or claims were made that substances like heteroauxin were responsible for the pathogenicity of crown gall bacteria, have been repeated. Although their results have been generally confirmed, their conclusions have appeared unjustified. So far as the writers are aware, the crown gall bacteria are pathogenic independently of auxin production.

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A STUDY OF SAMPLING IN CROSS-SECTION MEASUREMENTS OF WOOL FIBER [1]

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INTRODUCTION

Since the present wool-grading system is based largely on fiber diameter, standardization of the technique of sampling and measurement is of great importance to the industry. Various procedures are now employed for the measurement of wool-fiber diameter, including the cross-section method, which is recognized as an official method by the American Society for Testing Materials (*1, pp. 173–176*).[3]

In the previous paper (*2*) a discussion of methods which have been and are now in use in the United States and foreign countries was presented. It was pointed out that in the case of the conventional rapid cross-section method it was not possible to record the diameter at more than a limited number of points along the fiber length. This is in contrast to the method employed by the British Wool Industries Research Association and approved by the International Wool Textile Organization (*10*) in which the fibers are cut into very short lengths in order that the entire length of the fiber may be represented in the final sample measured.

Malan, Carter, and Van Wyk (*7*) have recently reported a study of various methods of sampling Merino wool fiber for diameter measurements. One set of fibers was cut transversely into as fine fragments as possible throughout the length of the fiber; the second set was cut transversely at three points (base, middle, and tip) along the fiber lengths; the third was cut transversely at the middle of the staple; and the fourth obliquely across the base and tip of two different sections of fiber. Euparal was used as the mounting medium and the diameters of the fibers as they fell onto the slide were measured microscopically by two different observers, the assumption being that the fibers will fall in such a manner that a large number of measurements will form an even distribution between greatest and least diameters represented in the sample. From a statistical standpoint the authors concluded that the third and fourth methods had much to recommend them, the fourth method being the most difficult sampling procedure. Significant differences between the measurements of the two observers were found. It was concluded that the measurement of 250 fibers on a single well-prepared slide is sufficient.

The present study was undertaken to determine the variation in fiber diameter and contour in samples of fiber from three positions

[1] Received for publication June 5, 1940. Paper No. 137 of the Journal Series, South Dakota Agricultural Experiment Station.

[2] The author wishes to express her gratitude to Dr. S. P. Swenson, associate agronomist for the South Dakota Agricultural Experiment Station, and to Dr. F. R. Immer, professor of agronomy and plant genetics, University of Minnesota, for assistance with the statistical analyses of the data; and to Barbara Webster, former student at South Dakota State College, for preparing the microscopic slides.

[3] Italic numbers in parentheses refer to Literature Cited, p. 415.

along the fiber length. It was hoped that the statistical analysis would indicate whether, in grading flocks of sheep, there is an advantage in measuring fiber diameter at more than one point along the fiber length and also whether, in the grading of individual samples of wool fiber, a significant difference in diameter and contour ratio exists at three specified points along the fiber length. The animals represented in this study were fed the same diet and maintained under the same environmental conditions.

METHODS AND MATERIALS

Samples of wool fiber from the Notail breed of sheep raised at the South Dakota Agricultural Experiment Station (*11*) were taken from the shoulder, middle, and thigh portions of nine individuals. Portions of the samples were then cut into three equal parts, and the fiber thoroughly cleaned in carbon tetrachloride, stained with a saturated solution of picric acid, and stored in a desiccator containing sulfuric acid of a concentration calculated to maintain an atmosphere of approximately 65 percent relative humidity. Approximately one-half of each sample portion was required to prepare the two slides for fiber measurements. The procedures used to prepare the microscopic slides and to secure the fiber diameter measurements of 200 fibers per sample have been described previously (*2*). In each instance the diameter reported is that found at approximately the middle of each sample portion of the fiber, but it is not possible in the cross-section method to record fiber diameter for each individual fiber used at the three points measured. All the measurements were made by a single observer.

The mean diameter and contour ratios were determined, and the standard deviation was calculated to furnish a measure of the variability. The formula suggested by Snedecor (*9*) has been used throughout in the chi-square test for homogeneity of errors, and in certain instances Bartlett's test for homogeneity (*4*, *8*) was applied. The analysis of variance developed by Fisher (*6*) has been applied throughout the study.

EXPERIMENTAL RESULTS

It is recognized in the analyses of the data used in this discussion that the nine fleeces employed do not necessarily characterize the Notail breed. These particular sheep were chosen for the study because it was believed that they exhibited certain superior fiber qualities and would be desirable animals to use for breeding purposes. It was felt, however, that the data obtained would be useful in suggesting certain fiber relationships that may exist in a flock of sheep and the advantages or disadvantages of the method used to study fiber diameter and contour. The latter information would be of particular value to the wool grader.

Location on the animal, such as shoulder, middle, and thigh, will be indicated by that designation, and the particular point on the fiber will be referred to as position in the fiber.

In table 1 are given the average diameters of 200 fibers from each of 3 locations on each of 9 sheep at 3 points along the fiber length. The standard deviation of single observations has been calculated to indicate the variability of these measurements.

TABLE 1.—*Average diameter and standard deviation of single measurements of 200 wool fibers from each of 3 positions in the wool fiber at 3 locations on the Notail breed of sheep* [1]

Location	Sample No.	Flesh		Midpoint		Tip		Average	
		Mean diameter	Standard deviation	Mean diameter	Standard deviation	Mean diameter	Standard deviation	Mean diameter	Standard deviation
		μ	μ	μ	μ	μ	μ	μ	μ
Shoulder	4190	31.58	6.10	31.82	5.66	30.35	5.60	31.25	5.79
	4630	28.40	4.64	30.36	4.94	31.42	5.01	30.06	4.86
	4671	26.62	6.13	27.28	5.88	26.77	5.25	26.89	5.77
	4813	26.77	3.98	27.84	3.56	28.61	3.29	27.74	3.62
	4901	23.46	4.25	24.86	4.23	24.38	4.20	24.23	4.23
	4914	29.12	4.65	29.33	4.81	28.88	4.65	29.11	4.70
	5151	25.32	4.09	26.10	4.51	23.74	4.04	25.05	4.22
	5304	28.98	4.80	30.52	5.28	30.06	4.91	29.85	5.00
	5412	24.79	4.46	24.85	4.93	25.30	4.16	24.98	4.53
	Average	27.23	4.84	28.11	4.91	27.72	4.61	27.68	4.79
Middle	4190	30.23	6.55	31.77	5.70	30.67	5.46	30.89	5.92
	4630	27.05	5.46	28.96	5.84	30.60	5.71	28.87	5.67
	4671	28.68	4.17	29.12	4.11	29.12	4.02	28.97	4.10
	4813	27.17	6.09	26.83	5.55	28.48	5.46	27.49	5.71
	4901	23.74	5.24	26.23	5.01	23.80	5.22	24.59	4.84
	4914	28.56	5.25	28.72	5.05	28.06	5.20	28.45	4.83
	5151	25.94	5.24	27.37	5.39	28.34	4.48	27.22	5.05
	5304	26.51	4.70	28.22	4.50	27.94	4.13	27.56	4.45
	5412	24.81	4.93	24.14	4.62	25.22	4.58	25.39	4.71
	Average	26.96	5.33	27.93	5.12	28.02	4.95	27.71	5.06
Thigh	4190	29.24	6.91	31.37	5.99	29.18	5.57	29.93	6.18
	4630	28.22	10.25	31.58	11.17	36.62	10.61	32.14	10.68
	4671	27.61	10.11	25.98	7.87	27.23	8.68	26.94	8.93
	4813	32.82	6.37	35.00	6.82	35.88	5.58	34.57	6.28
	4901	27.17	8.33	27.40	8.10	27.78	8.50	27.45	8.31
	4914	30.28	7.67	30.44	6.44	31.90	7.22	30.87	7.13
	5151	27.80	7.34	28.06	6.90	30.22	6.71	28.69	6.99
	5304	34.90	8.86	34.38	7.87	33.82	7.89	34.37	8.22
	5412	30.84	10.30	27.32	6.88	30.88	6.83	29.68	8.17
	Average	29.88	8.58	30.17	7.70	31.50	7.66	30.52	7.99

[1] All average standard deviations in this table were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root.

If a composite is made of the measurements of the fiber in the three positions, considerable variation will be found to exist in fiber diameter between sheep-within-fiber-position at any given location (table 1). On an average the standard deviation of the fiber at the thigh of the fleeces was greater than at the middle and shoulder. The middle and shoulder showed approximately the same variability in standard deviation among the three portions of fiber within each location.

As shown in table 2, with few exceptions the ratio of the greatest diameter of the fiber to the diameter at right angles to it was greater than the 1.20 : 1 ratio reported by Barker (*3*) for the ideal fiber. With the exception of 1 middle and 11 thigh samples all contour ratios were between ratios of 1.2 : 1 and 1.3 to 1, the average ratio for wool fiber computed by Von Bergen and Krauss (*5*). At each position in the fiber the average contour ratios increased progressively from the shoulder to the middle to the thigh locations on the fleeces, and the standard deviations showed the same tendency.

The chi-square test for homogeneity of errors has been used to determine whether the variability of the fiber diameter and contour measurements for separate locations on the fleeces and among the

various fiber positions were homogeneous (*9*). The Bartlett test was applied in all the diameter measurements at the shoulder location on the fleeces, and in no case did the values obtained change the significance of the errors. Therefore, the results with Snedecor's method are used throughout the present discussion.

TABLE 2.—*Average contour ratios and standard deviations of single measurements of 200 wool fibers from each of 3 positions in the wool fiber at 3 locations on the Notail breed of sheep* [1]

Location	Sample No.	Flesh		Midpoint		Tip		Average	
		Mean fiber-contour ratio	Standard deviation	Mean fiber-contour ratio	Standard deviation	Mean fiber-contour ratio	Standard deviation	Mean fiber-contour ratio	Standard deviation
Shoulder	4190	1.24	0.141	1.29	0.195	1.25	0.160	1.26	0.167
	4630	1.21	.114	1.21	.112	1.25	.130	1.22	.119
	4671	1.25	.142	1.24	.146	1.26	.155	1.25	.148
	4813	1.24	.133	1.22	.139	1.25	.153	1.24	.142
	4901	1.26	.150	1.27	.149	1.27	.182	1.27	.160
	4914	1.22	.133	1.28	.143	1.25	.157	1.25	.145
	5151	1.24	.146	1.24	.153	1.24	.158	1.24	.152
	5304	1.22	.126	1.23	.136	1.28	.146	1.24	.136
	5412	1.20	.127	1.23	.167	1.23	.123	1.22	.140
	Average	1.23	.135	1.24	.150	1.25	.152	1.24	.146
Middle	4190	1.25	.145	1.29	.162	1.28	.163	1.27	.157
	4630	1.23	.132	1.25	.148	1.25	.151	1.24	.144
	4671	1.30	.181	1.28	.183	1.27	.166	1.28	.177
	4813	1.25	.151	1.27	.142	1.27	.163	1.26	.152
	4901	1.25	.139	1.31	.169	1.26	.154	1.27	.154
	4914	1.27	.150	1.27	.149	1.28	.158	1.27	.152
	5151	1.24	.144	1.28	.172	1.30	.204	1.27	.175
	5304	1.22	.150	1.21	.117	1.26	.142	1.23	.137
	5412	1.23	.148	1.24	.140	1.25	.156	1.24	.148
	Average	1.25	.149	1.27	.155	1.27	.163	1.26	.156
Thigh	4190	1.30	.165	1.31	.190	1.28	.155	1.30	.171
	4630	1.26	.186	1.29	.163	1.33	.196	1.29	.182
	4671	1.33	.206	1.28	.189	1.29	.173	1.30	.190
	4813	1.35	.184	1.33	.203	1.36	.214	1.35	.201
	4901	1.29	.176	1.31	.197	1.29	.187	1.30	.187
	4914	1.30	.190	1.35	.192	1.33	.174	1.33	.186
	5151	1.28	.163	1.28	.154	1.29	.187	1.28	.168
	5304	1.29	.195	1.31	.204	1.30	.168	1.30	.189
	5412	1.30	.201	1.24	.142	1.31	.195	1.28	.181
	Average	1.30	.186	1.30	.183	1.31	.184	1.30	.184

[1] All average standard deviations in this table were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root.

TABLE 3.—*Values of χ^2 found from homogeneity tests of variance pertaining to diameter and contour measurements of fiber at different locations on fleece* [1]

Location on fleece	Degrees of freedom	χ^2 for—					
		Diameter			Contour		
		Flesh	Midpoint	Tip	Flesh	Midpoint	Tip
Shoulder	8	97.53	57.55	96.78	21.16	28.74	20.22
Middle	8	70.42	40.39	160.11	78.36	51.26	44.69
Thigh	8	64.74	48.24	152.22	40.60	41.78	29.91

[1] All χ^2 values in this table exceed 1-percent point.

Table 3 shows the values of χ^2 for a homogeneity test of errors for the diameter and contour measurements when a composite is made of all the fleeces at any one fleece location and position. In the case of the diameter measurements all the composites were heterogeneous. The flesh and midpoint samples were more heterogeneous at the shoulder, and the tip at the middle and thigh locations. The most homogeneous sample was the middle midpoint and the most heterogeneous the middle tip.

In the case of the contour measurements (table 3), all the composites were heterogeneous. In most of the contour measurements the samples did not exhibit as great a tendency to heterogeneity as the diameter measurements.

If the fiber data are grouped according to location on a fleece and χ^2 is computed for the relationship between fiber positions, homogeneity will be found to exist in approximately three-fourths of the diameter measurements (table 4). Thus in all but two fleeces at the shoulder location, two at the middle, and three at the thigh, homogeneity existed between the flesh, midpoint, and tip portions of the fiber of the same fleece.

TABLE 4.—*Values of χ^2 found from homogeneity tests of variance pertaining to diameter and contour measurements of fiber from different fleeces* [1]

Fleece No.	Degrees of freedom	χ^2 for—					
		Diameter			Contour		
		Shoulder	Middle	Thigh	Shoulder	Middle	Thigh
4190	2	2.88	*7.84	3.28	**24.52	3.29	**9.52
4630	2	1.25	.93	1.51	5.92	3.34	*6.89
4671	2	4.79	.28	**13.04	1.68	2.24	*6.13
4813	2	*7.43	2.97	*7.70	4.14	3.90	4.49
4901	2	.02	.49	.48	**11.42	*7.14	2.55
4914	2	.31	.32	5.93	5.45	.73	2.18
5151	2	3.08	*6.99	1.76	1.30	**23.00	*8.21
5304	2	*8.56	3.31	3.92	4.38	**11.82	*7.42
5412	2	5.98	1.35	**52.13	**25.38	2.88	**22.71

[1] *χ^2 exceeds 5-percent point; **χ^2 exceeds 1-percent point.

As shown in table 4, diameter was more homogeneous throughout the fiber length than contour ratio. Fiber that was homogeneous in diameter measurements was not necessarily homogeneous in contour.

Homogeneity of errors is assumed in the calculations of the analysis of variance, but, as was shown in tables 3 and 4, the individual fleeces and composites did not exhibit this characteristic in all instances. Since, however, the nine fleeces studied were the only ones for which data were available, the analysis of variance was applied, recognizing that the variability of the individual fiber measurements of the different sheep was not of a homogeneous nature.

In table 5 are shown the mean squares for sheep, positions in the fiber, and the interaction of sheep $\times$ position (error) of the diameter and contour measurements. Generalizations regarding average diameters and contours for the three positions in the fiber over the entire fleece and for all sheep of the breed may be made from the data in this table. In the case of the diameter measurements, differences between sheep measurements at a single location were highly significant, and differ-

ences due to position in the fiber were significant at the middle location only. The mean square value for variation due to error was lowest at the middle location and highest at the thigh.

In the contour ratios reported in table 5, a significant difference between means of fiber measurements at the middle location was found, while all other differences were nonsignificant. The error variance for the thigh location was approximately twice that of the shoulder and middle.

TABLE 5.—*Mean squares for sheep, position in the fiber, and error from analyses of variance of diameter and contour measurements made at three locations on the fleece* [1]

Location	Mean-square values for sheep,[2] position,[3] and error [4] at 3 locations					
	Diameter			Contour		
	Sheep	Position	Error	Sheep	Position	Error
Shoulder	**3,726.91	315.01	204.78	0.1351	0.2423	0.0675
Middle	**2,412.19	*614.23	161.83	*.2239	.1819	.0668
Thigh	**4,542.76	1,382.26	575.22	.2696	.0536	.1280

[1] *Exceeds 5 percent point in the *F* test; **exceeds 1 percent point in the *F* test.
[2] 8° of freedom.
[3] 2° of freedom.
[4] 16° of freedom.

TABLE 6.—*Mean squares for position in fiber and error at three locations from analyses of variance of fiber diameter and contour measurements made for the Notail breed* [1]

Location	Position in fiber	Mean-squares for sheep [2] and error [3] in 3 positions at 3 locations			
		Diameter		Contour	
		Sheep	Error	Sheep	Error
Shoulder	Flesh	**1,282.75	23.53	**0.0900	0.0182
	Midpoint	**1,302.75	24.11	**.1605	.0176
	Tip	**1,550.75	21.12	.0389	.0229
Middle	Flesh	**800.50	28.46	**.1189	.0224
	Midpoint	**917.25	26.12	**.1944	.0238
	Tip	**1,024.38	24.53	.0486	.0266
Thigh	Flesh	**1,366.12	166.63	**.1626	.0344
	Midpoint	**2,058.88	59.22	**.3864	.0327
	Tip	**2,263.25	58.72	**.1405	.0337

[1] **Exceeds 1 percent point in the *F* test.
[2] 8° of freedom.
[3] 1,791° of freedom.

Table 6 shows the application of the analysis of variance to the breed composite of the three positions in the fiber at the three locations on the sheep. Separation of the data by position in the fiber for the three locations in the composited fleeces is of importance in indicating whether choice of the position of measurement of diameter and contour is necessary or of significance in fleece grading. Variation due to sheep was found to be highly significant in all cases of diameter measurements, and in all but two cases of contour measurements. The mean-square values due to error were relatively constant for the three positions at the shoulder and middle locations for the diameter

measurements, while the value for the flesh thigh was almost three times that of the midpoint or tip thigh.

When the analysis of variance was applied to the diameter studies of variation due to position in the fiber within a single location on a fleece, differences were found to be significant at the 1-percent point in 15 instances, significant at the 5-percent point in 4 instances, and non-significant in 8 instances. These results are shown in table 7. In the majority of fleeces studied the mean-square values for variation due to error were appreciably higher at the thigh location than at either the shoulder or the middle locations. The range in significance found for the specific positions within locations on a fleece indicates that the use to which the data would be put will be the deciding factor in sampling fleeces for analysis.

TABLE 7.—*Mean squares for position in the fiber and error from analyses of variance of fiber diameter measurements made at 3 locations on the fleece* [1]

Fleece No.	Mean squares for position in fiber [2] and error [3] at 3 locations					
	Shoulder		Middle		Thigh	
	Position	Error	Position	Error	Position	Error
4190	*124.5	32.86	*126.0	35.03	**312.0	38.20
4630	**469.5	23.71	**633.0	32.17	**3,575.0	114.08
4671	23.0	33.23	13.5	16.82	146.5	79.86
4813	**171.0	13.13	**150.0	32.39	**498.5	39.43
4901	**101.0	17.88	**405.0	26.59	*269.5	68.20
4914	9.5	22.13	24.0	26.74	*161.5	50.84
5151	**289.5	17.81	**290.5	25.56	**353.5	49.81
5304	**125.0	25.02	**167.5	19.78	59.0	67.61
5412	15.0	20.50	58.5	22.22	**833.0	66.73

[1] *Exceeds 5 percent point in the *F* test; **exceeds 1 percent in the *F* test.
[2] 2° of freedom.
[3] 597° of freedom.

TABLE 8.—*Mean squares for position in the fiber and error from analyses of variance of fiber contour measurements made at 3 locations on the fleece* [1]

Fleece No.	Mean squares for position in fiber [2] and error [3] at 3 locations					
	Shoulder		Middle		Thigh	
	Position	Error	Position	Error	Position	Error
4190	**0.1763	0.0266	0.1030	0.0989	0.0219	0.0291
4630	**.1172	.0144	.0152	.0204	**.3021	.0328
4671	.0269	.0218	.0566	.0315	*.1572	.0363
4813	.0300	.0204	.0321	.0234	.0644	.0401
4901	.0322	.0262	**.1793	.0240	.0200	.0342
4914	**.1546	.0208	.0262	.0231	*.1584	.0343
5151	.0220	.0230	**.1752	.0304	.0178	.0285
5304	**.1907	.0185	**.1271	.0187	.0306	.0357
5412	*.0709	.0198	.0356	.0221	**.2957	.0329

[1] *Exceeds 5 percent point in the *F* test; **exceeds 1 percent point in the *F* test.
[2] 2° of freedom.
[3] 597° of freedom.

In table 8 an analysis of variance is applied to the contour measurements at the three positions in the fiber at a single location on individual sheep. Variation due to position within a single location for contour on a fleece was highly significant in nine instances and

significant in three instances. In only three of the fleeces were significant differences found at the middle location on a fleece. With two exceptions variation due to error was higher at the thigh than at either the shoulder or middle location on individual sheep.

Further justification for the application of the analysis of variance where heterogeneity has been shown may be seen by examination of the data in the tables presented above. Using measurements at the shoulder location as an example, while the variances between fibers within individual sheep were heterogenous, the variances for individual sheep ranged from 13.13 to 33.23 (table 7), all numerically small as compared with the variance due to sheep×position (204.78). There can be little question that the variance between sheep is highly significant. The range for individual sheep (13.13 to 33.23) while significant because of the large number of measurements involved, is relatively unimportant because of its small influence in changing the value of the interaction.

SUMMARY AND CONCLUSIONS

Since sampling of wool fleeces, preparation of microscopic slides, fiber measurements, and the necessary calculations are all time-consuming procedures, the present study was undertaken to determine whether in wool-breeding programs the measurement of fiber diameter and contour at more than one point along the fiber length is of importance. The samples of wool were taken from different locations on fleeces of sheep of the Notail breed, exposed to the same environmental conditions and given the same feedstuffs.

Examination of the analysis of variance indicates, in general, that significant differences in diameter of fiber existed between the various sheep of the same breed and that in some fleeces significant differences in the measurements of diameter and contour at various points along the fiber length were present.

Homogeneity of fiber diameter and contour measurements did not exist between fleeces within the breed studied and within positions in the fiber at three locations on the same sheep. Homogeneity was found in approximately three-fourths of the instances when the χ^2 test was applied to studies of wool from the three fiber positions at a single location upon individual sheep, and in more than one-half of the contour measurements of the same grouping.

Applications of the analysis of variance to fleeces and fleece components indicated significant differences involving diameter measurements because of variation due to location on sheep (shoulder, middle, or thigh); variation due to position in the fiber at the middle location; variation due to sheep at the three fiber positions for each fleece location in all instances; and variation due to position in the fiber for approximately two-thirds of the individual fleeces at each location. In the contour studies significant differences were found because of variation due to sheep at the middle location; variation due to sheep in all but two instances of positions at each location; and variation due to position in less than half of the fleeces at each location.

From a practical standpoint, the measurement of fiber diameter and contour at all three positions used in the present study would not seem to be necessary in a breeding program where such measurements are used in the preliminary elimination of undesirable or variable animals from the flock. Examination of data obtained by measure-

ments at a single position upon fibers at three locations on the fleeces would indicate whether appreciable variation among locations existed. These same data could be used in comparisons of sheep within a flock.

From the results here reported it may be concluded that in studies of the effects of environment and feed, consideration of variation throughout the length of the wool fiber is of importance. Spinnability of wool is effected by contour and diameter and their variation among and within fibers. Thus, in conducting a wool-breeding program to produce a maximum of desirable fleece qualities, this study indicates that diameter and possibly contour should be measured at more than one point.

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ROOT ROT OF PEPPER AND PUMPKIN CAUSED BY PHYTOPHTHORA CAPSICI [1]

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INTRODUCTION

A destructive root rot of bell or sweet green pepper (*Capsicum annuum* L. var. *grossum* Sendt.) and pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) plants was observed in a truck section of the San Joaquin Valley, near Merced, Calif., on May 19, 1934 (*7*).[2] In addition to a high mortality, many plants showed sudden and severe wilting, and as the season progressed, there was a marked reduction in stand. The disease was caused by *Phytophthora capsici* Leonian (*5*). Because of the economic importance of these crops, an investigation of the disease was undertaken. The results are presented herewith.

REVIEW OF LITERATURE

In 1922, Leonian (*5*) described a stem and fruit blight of chili pepper, caused by *Phytophthora capsici* sp. nov., which was prevalent in New Mexico. Symptoms of the disease consisted mainly of dry lesions on the pods, blighting of young and older branches, and girdling. While the pathogen did not cause a root rot under field or greenhouse conditions, it sometimes affected some of the younger roots. Introduction of the fungus into the soil failed to cause wilting of healthy pepper plants.

Subsequently, the blight phase of the disease was reported from other States and foreign countries. There are, however, several reports dealing with the root rot phase which are herewith reviewed because of their direct bearing on this paper.

Wilt and desiccation of chili and eggplant seedlings, accompanied by a collar constriction, was recorded in the province of Naples, Italy, in 1924 by Trotter (*11*). The flowers and foliage were not directly affected. The causal organism was identified as *Phytophthora omnivora.* This appears to be the first record of the occurrence of a phytophthora disease of pepper characterized by wilting due to root infection.

In 1927, Curzi, (*1*) described a root rot disease of chilies caused by *Phytophthora hydrophila.* There was evidence that the disease

[1] Received for publication February 4, 1941. Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station, and the Department of Botany, Missouri Agricultural Experiment Station (Journal Series No. 720).

[2] Italic numbers in parentheses refer to Literature Cited, p. 425.

was spread by water. Tucker (*12*) has since shown that this species is identical with *P. capsici.*

Sarejanni (*9*) found a serious collar rot of chilies in Macedonia, Greece, in 1933, which was caused by *Phytophthora capsici.* At least 70 percent of the crop was affected. Diseased plants quickly wilted, while occasional attacks were noted on the fruits and on young plants in seedbeds. Infection was favored by heavy watering and the prevailing high summer temperatures.

In Florida, in 1932, Weber (*13*) observed both a blighting of the aerial parts (stems, branches, fruit, and leaves) and a girdling of pepper plants at the soil level by *Phytophthora capsici*, resulting in sudden wilting and death. The latter symptom may have been caused by root infection, although it is not so stated.

According to Kreutzer (*3*), a species of *Phytophthora* (probably *P. capsici*) isolated from rotting cucumber fruits caused severe damping-off of cucumber and pepper seedlings. Also, 10 to 20 days after the fungus was introduced into soil in which mature pepper plants were growing, the plants developed a severe blight.

In a recent annual report of the Colorado Agricultural Experiment Station (*8*), is was stated that *Phytopthora capsici*, obtained from a fruit rot of tomato, had been shown to cause a wilt of pepper plants. In Weld county, the same organism was responsible for a wilt of squash and watermelon.

The results of further studies on a decay of cucumber and tomato fruits and on a wilt of squash and watermelon vines, caused by *Phytophthora capsici*, were recently published by Kreutzer, Bodine, and Durrell (*4*).

Tompkins and Tucker (*10*) showed that an isolate of *Phytophthora capsici* from a honeydew melon caused wilting of young pepper plants in the greenhouse within 10 days after its introduction into the soil, and after 20 days all were badly wilted and dying.

Godoy (*2*) in a recent paper mentioned the occurrence of damping-off of pepper seedlings in Argentina.

FACTORS FAVORING INFECTION

Pepper and pumpkin plants require abundant soil moisture, good drainage, and relatively high air temperatures for optimum growth. By the same token, these are the principal environmental factors which favor inception and spread of the root rot disease in the truck sections near Merced. In most cases, these crops are grown along river bottoms and in low areas which, although rich in fertility, lack adequate drainage. With frequent, heavy, surface irrigation, necessitated by the growth requirements of the plants, the soil quickly becomes excessively wet and often waterlogged.

It has been observed that when plants are grown on higher, well-drained ground and supplied with only enough moisture to satisfy growth requirements, then the incidence of disease is greatly reduced.

SYMPTOMS OF THE DISEASE

Field observations and greenhouse tests under controlled conditions indicated that infection of pepper and pumpkin plans by *Phytophthora capsici* results in symptoms common to both. Although a few

plants die early in the season when temperatures are naturally lower, a high incidence of disease is uncommon until the advent of high temperatures at midseason, at which time many plants have reached the fruiting stage. Infection continues on a high level during the rest of season. The disease appears very suddenly, and infected plants usually die promptly.

Above ground, the chief diagnostic symptoms of the disease consist of a rapid, permanent wilting of the leaves, without noticeable change in color; a blackish-brown discoloration of the lower part of the stem, adjacent to the soil level; and collapse of the stem, followed by lodging of the plant on the ground.

Under ground, the invaded roots and stems show a soft, water-soaked, blackish-brown, odorless type of decay which ultimately involves all tissues. Diseased plants are easily pulled from the soil, but invariably the cortex of the taproot and lateral roots sloughs off and remains behind.

THE CAUSAL FUNGUS, PHYTOPHTHORA CAPSICI

ISOLATION

The causal fungus, *Phytophthora capsici*, was readily isolated from the roots and basal part of the stems of recently infected pepper and pumpkin plants by removing aseptically small tissue fragments from the internal, advancing margin of decay, planting on either prune or malt-extract agar (*6*), and incubating at room temperature. Also, small sections of fine lateral roots, after thorough washing, were planted directly on agar. After 48 hours, pure cultures of the fungus were established on agar slants by transferring hyphal tips from the edge of the Petri-dish colonies. The fungus was cultured at intervals from several lots of naturally infected specimens, involving about 100 pepper and 50 pumpkin plants. Pure cultures of *P. capsici* were constantly obtained from tissue plantings, and there was no indication of the presence of other species.

IDENTITY AND TEMPERATURE RELATIONS

Comparative studies were made on three isolates from Yellow Crookneck pumpkin, one from Summer pumpkin, and two from bell pepper (var. Barbanera no. 1). All isolates were secured from the roots and lower portions of stems of diseased plants in the Merced area. The isolates exhibited the usual slight variations in type and rapidity of growth in agar plates, in tendency to develop sporangia and oogonia, and in size of reproductive organs. The isolates from pumpkin could not be distinguished from those from pepper.

The sporangia were papillate, limoniform to irregular or elongate, with a mean size of 41 by 30 microns when produced in oatmeal-agar cultures. In liquid cultures the sporangia were somewhat larger and produced zoospores more frequently than when developed on agar media. Chlamydospores were not observed, but in oogonium-producing cultures occasional unfertilized oogonia germinated by germ tubes in the manner characteristic of chlamydospores.

Oogonia and oospores were formed in large numbers by one isolate, while two failed to develop them even after 3 months' incubation

at 15° C. The remaining three isolates developed a few oogonia and oospores in old cultures. The antheridia were of the permanent, amphigynous type, encircling the stalk of the oogonium.

The temperature-growth relations of the isolates were observed after 96 hours' growth on Difco corn-meal agar plates (pH 6.1). All grew well at temperatures ranging from 15° to 35° C. The optimum temperature appeared to lie between 25° and 30°, two isolates showing slightly greater growth at the former and four at the latter temperature. Minor differences were observed in growth rates, and in the appearance of the mycelial weft, which varied from regularly radiate growth to a matted, slightly irregular type. Some isolates exhibited both types of growth when incubated at different temperatures.

The isolates from pumpkin and pepper corresponded very well in all characters with isolates of *Phytophthora capsici* from pepper studied under comparable conditions by Tucker (*12*), with isolates from honeydew melons reported by Tompkins and Tucker (*10*), and with isolates from Winter Queen watermelons studied by Wiant and Tucker (*14*).

PATHOGENICITY TESTS

Pure cultures of *Phytophthora capsici*, isolated from the roots of naturally infected Barbanera No. 1 pepper and Early White Bush Scallop, Zucchini, and Yellow Crookneck pumpkin plants, were used in the infection experiments. Inoculum was prepared by growing the pepper and pumpkin isolates of the fungus on sterilized, moistened cracked wheat. It was added in uniform quantity to autoclaved soil in 6-inch pots, each containing a young plant, in a manner designed to avoid wounding the roots. Sterilized wheat was used for the noninoculated controls. The plants, grown in a greenhouse at temperatures ranging from 28° to 32° C., were watered heavily each day to provide favorable conditions for infection.

On Pepper

The pathogenicity of three pepper isolates was proved on the variety Barbanera No. 1, because this pepper is grown extensively in the Merced section. On July 18, 1934, 25 pepper plants were inoculated with each isolate. By August 1, all plants were infected, the incubation period ranging from 6 to 14 days. Symptoms shown by diseased plants were identical with those resulting from natural infection (fig. 1, *A*). Typical wilting of the leaves, destruction of the root system, and browning of the basal part of the stem with lodging of the plant occurred. When diseased plants were pulled from the soil, the soft, watery, blackish-brown cortical tissues sloughed off from the central cylinder of the taproot (fig. 2, *A*). Most of the lateral roots were completely rotted, in marked contrast to the healthy condition (fig. 2, *B*). The 15 noninoculated controls continued healthy (fig. 1, *B*). All infected roots yielded the fungus when plated.

On August 17, a total of 60 pepper plants of the same variety were inoculated with the reisolates. All plants were infected by August 27, the incubation period ranging from 5 to 10 days. The 10 noninoculated controls remained healthy.

Damping-off of Barbanera No. 1 pepper seedlings occurred when seeds were planted in previously infested soil or in autoclaved soil to which the fungus isolates were later added.

On Pumpkin

The pathogenicity of the pumpkin isolates was proved on the varieties Early White Bush Scallop, Zucchini, and Yellow Crookneck, the seeds of which had been obtained from a grower in the Merced section. On August 10, 1934, 25 pumpkin plants of each variety were inoculated with each isolate. By August 31, all plants were infected, the incubation period ranging from 12 to 21 days. The symptoms shown by diseased plants were identical with those resulting from natural infection and followed quite closely the sequence described for artificially infected pepper plants. The fungus was reisolated from

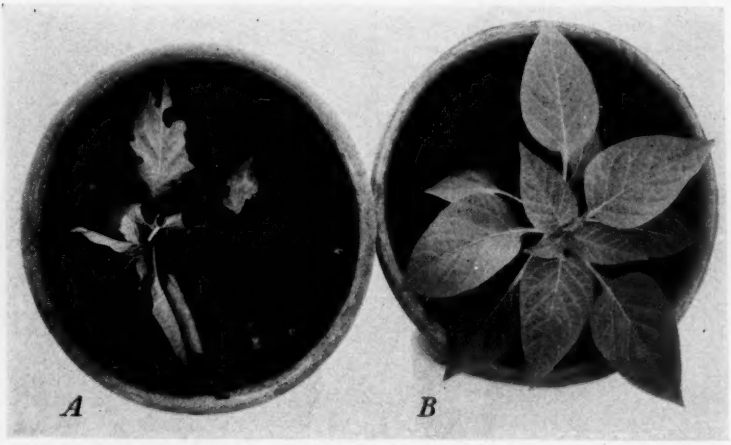

FIGURE 1.—Phytophthora root rot of pepper and pumpkin: *A*, Artificial infection of the pepper variety Barbanera No. 1 after 8 days, showing rapid wilting and collapse of the plant; *B*, healthy control.

all infected plants. The five noninoculated controls of each variety remained healthy.

On September 15, 20 pumpkin plants of each of the four varieties were inoculated with the reisolates, and all plants became diseased by September 25. The incubation period ranged from 5 to 10 days. The noninoculated controls remained healthy.

Similarly, damping-off of Early White Bush Scallop, Zucchini, and Yellow Crookneck pumpkin seedlings occurred, as mentioned above for pepper.

Cross Inoculations

In parallel inoculations, three isolates of *Phytophthora capsici* from pepper proved pathogenic to Early White Bush Scallop, Zucchini, and Yellow Crookneck plants in the greenhouse, and, likewise, two isolates each from Early White Bush Scallop, Zucchini, and

FIGURE 2.—Phytophthora root rot of pepper and pumpkin: *A*, Blackening of the stem and decay of the root system of young Barbanera No. 1 pepper plant; *B*, healthy control.

Yellow Crookneck pumpkin caused infection of pepper plants (fig. 3), with incubation periods closely corresponding to those just mentioned. It may be assumed, therefore, that these isolates, based upon their infective capacity, are very similar, if not identical.

These isolates were also tested by inoculating healthy Zucchini pumpkin and pepper fruits in the laboratory, using the technique described elsewhere (*10*). All isolates induced a severe type of fruit rot.

SUSCEPTIBILITY OF PEPPER AND PUMPKIN VARIETIES TO INFECTION

Numerous commercial varieties of pepper and pumpkin were tested for susceptibility to infection with their respective isolates of *Phytophthora capsici*. No fewer than 20 plants of each variety were inoculated according to the procedure previously described, involving in the aggregate about 2,500 pepper and 300 pumpkin plants. All varieties proved to be highly susceptible, with no survival of individuals in any particular variety. The incubation period for pepper varieties ranged from about 5 to 49 days; for pumpkin varieties, from 4 to 33 days. When considered collectively, the minimum incubation period for pepper varieties was 8 days and the maximum 29 days; for pumpkin plants, the minimum was 8 days and the maximum 23 days.

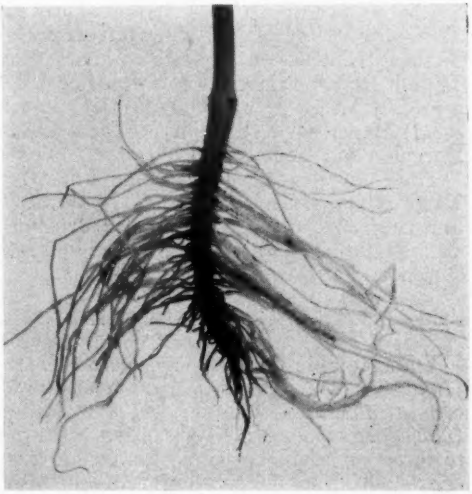

FIGURE 3.—Phytophthora root rot of pepper and pumpkin. Artificial infection of young pepper plant, after 16 days, with an isolate of *Phytophthora capsici* from Zucchini pumpkin, showing a blackish-brown discoloration of the basal part of the taproot and some lateral roots.

In the winter of 1937, seeds which had been collected at Merced from apparently resistant Barbanera No. 1 pepper plants were planted in autoclaved soil in the greenhouse. The progeny proved highly susceptible upon inoculation.

The following varieties of pepper were tested in the greenhouse: Anaheim, Asgrow King, Bell or Bull Nose Hot, Bell or Bull Nose Sweet, Birdseye or Coral Gem, Black Long of Mexico, Bountiful No. 7, Bulgarian, California Wonder, Cayenne, Cayenne Large Thick, Cayenne Long Narrow, Celestial, Cherry, Chinese Giant, Colossal, Crimson Giant, Dwarf Chili Red Cluster, Early Giant, Early Mountain, Elephant Trunk, Floral Gem, Giant of China Golden Yellow, Giant of China Red, Gloria, Golden Dawn, Golden Queen, Green

Mexican Pickling, Green Pointed Sharp, Harris Earliest, Harris Early Giant, Heiffer Horn, Hercules, Hungarian Hot or Banana, Hungarian Yellow Wax, Kaleidoscope, Kalinko, King of the North, Large Bell or Bull Nose, Large Early Neapolitan, Large Long Red Cayenne, Large Mexican Chili, Long Hot or Finger, Long Podded, Long Red Cayenne, Mexican Chili, Mile High Sweet Giant, Monstrous, Narrow Podded Neapolitan, Neapolitan Giant, New Victory, Nikko Togarashi, Nocera Golden Yellow, Nocera Large Red, Nordico, Ohio Crimson Giant, Oshkosh, Pimiento, Prince of Wales, Prizetaker, Procopp's Giant Red, Proteus, Red Cherry, Red Chili, Red Cluster, Red Hercules, Rosse, Royal King, Ruby Giant, Ruby King, Ruby King Special, Spanish Bull, Spanish Gem, Square Yellow Big of Naples, Squash or Tomato Hot, Squash or Tomato Shaped, Squash or Tomato Sweet, Sudanese Chili, Sunnybrook, Sunnybrook Sweet Cheese, Sweet Meat Glory, Sweet Mountain or Mammoth, Sweet Salad Tomato, Sweet Spanish, Tabasco, Taka-No-Tsume, Tomato or Squash, Tomato Shaped Red Squash, Tom Thumb Round Scarlet, Verde, Verticus, White Stump Sweet, Windsor A, B, C, D, E, and G (selections from the Connecticut Agricultural Experiment Station), World Beater, Yatsu-Fusa, Yellow Hercules, and Yellow Oshkosh.

The following varieties of pumpkin were tested in the greenhouse: Early Green Bush Scallop, Early White Bush Scallop, Early Yellow Bush Scallop, Giant Summer Crookneck, Italian Marrow Cocozelle, Italian Marrow Zucchini, Mammoth Summer Crookneck, Table Queen, and Yellow Summer Crookneck.

EXPERIMENTAL HOST RANGE

Young plants of squash (*Cucurbita maxima* Duchesne), eggplant (*Solanum melongena* L. var. *esculentum* Nees), tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey), and Turkish tobacco (*Nicotiana tabacum* L.) were tested in the greenhouse for susceptibility to infection with the pepper and pumpkin isolates of *Phytophthora capsici*, using the inoculation technique previously described. A suitable number of noninoculated controls was reserved for each variety tested, and during the experiments they continued healthy. Reisolations were made from all diseased specimens, and the reisolated fungus from a particular host was then tested by inoculation into healthy specimens of that host.

The following varieties of squash proved highly susceptible: Banana, Blue Hubbard, Boston Marrow, Delicious, Delicious Golden, Golden Hubbard, Hubbard, and Warted Hubbard.

Of the five varieties of eggplant used, only two were shown to be susceptible, i. e., Black Beauty and Large New York Purple. No infection was obtained on Colossal, Florida High Bush, and Improved Large Purple.

Only one variety of tomato (Early Santa Clara Canner) was tested, and it proved highly susceptible.

None of the isolates caused infection of Turkish tobacco plants.

Tubers of potato (*Solanum tuberosum* L.) var. Russet Burbank were inoculated with pure cultures of the five isolates in the laboratory, but they remained healthy.

SUMMARY

A root rot of pepper and pumpkin plants, caused by *Phytophthora capsici* Leonian, is prevalent in the San Joaquin Valley, near Merced, Calif.

Excessive moisture, inadequate soil drainage, and high air temperatures definitely favor the inception and spread of the disease.

The root system and the basal part of the stems of pepper and pumpkin plants show a soft, wet decay. Permanent wilting of the leaves occurs very suddenly, with ultimate collapse of the stem and lodging and death of the plants.

The isolates of the fungus from pepper and pumpkin are described.

Infection was obtained in the greenhouse by adding the fungus to the wet, autoclaved soil of potted plants. The incubation period ranged from 6 to 14 days for pepper and from 12 to 21 days for pumpkin plants.

Successful cross inoculations were made with pepper isolates to pumpkin, and vice versa. All isolates caused damping-off of pepper and pumpkin seedlings in the greenhouse and rotting of pepper and pumpkin fruits in the laboratory.

The pepper and pumpkin isolates of *Phytophthora capsici* were also pathogenic to squash, eggplant, and tomato plants.

No resistance to the disease was found in any of the varieties of pepper and pumpkin tested under greenhouse conditions.

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THE CHEMICAL COMPOSITION AND APPARENT DIGESTIBILITY OF NUTRIENTS IN SMOOTH BROMEGRASS HARVESTED IN THREE STAGES OF MATURITY [1]

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INTRODUCTION

Smooth bromegrass (*Bromus inermis* Leyss.), a very leafy, sod-forming, long-lived perennial, ranks high among the cultivated grasses in palatability and yielding qualities. It requires more moisture for growth than crested wheatgrass (*Agropyron cristatum* (L.) Beauv.) and should not be considered a competitor of this grass in the drier areas. Because of its importance in pure stands and in mixtures with alfalfa and other legumes and grasses, both as a pasture and a hay crop, it seemed advisable to undertake a study of the chemical life history and the digestibility of the nutrients at various stages of maturity of this grass when grown under the soil and climatic conditions of the Palouse area of eastern Washington.

REVIEW OF LITERATURE

Smooth bromegrass has persisted for 20 years in a horse paddock at the Illinois Agricultural Experiment Station (*1*).[2] At the same station (*2*, *5*) 470-pound calves consumed 30 pounds of green grass containing 28.3 percent of dry matter and 850-pound steers consumed 52.7 pounds of the same grass daily. Smooth bromegrass in these studies contained 1,023 calories of digestible energy per pound of dry matter, and with a 68.29-percent availability, the net energy value was 699 calories. Field records at the Illinois Station (*1*) show smooth bromegrass to excel timothy, orchard grass, bluegrass, and reed canary grass in carrying capacity for yearling Hereford heifers. The gains produced during the first month of grazing were greater than those made during the remainder of the grazing season.

Under western dry-land conditions smooth bromegrass pasture does not produce the gains obtained from crested wheatgrass pasture. Orcutt (*7*) at the Moccasin substation in Montana recorded gains of 61 pounds per acre on crested wheatgrass pasture and 41.5 pounds on smooth bromegrass pasture when steers were grazed in 23.6-acre plots at the rate of four to eight steers per plot. When the value of old native grass pasture was rated as 100 percent, seedings of smooth bromegrass and crested wheatgrass had values of 112 and 177 percent, respectively.

[1] Received for publication March 3, 1941. Published as Scientific Paper No. 486, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[2] Italic numbers in parentheses refer to Literature Cited, p. 431.

Shepperd (*9*) at the North Dakota Experiment Station reported daily gains of 1.86 pounds per head when steers were grazed on second-year sweetclover as compared with gains of 1.54 pounds on smooth bromegrass pasture.

Studies by Hopper and Nesbitt (*3*) of the more mature stages of smooth bromegrass showed a protein content of 8.80 percent at blossoming time and a crude fiber content of 29.96 percent. Before heading it contained 15.14 percent protein and 23.56 percent crude fiber. Ladd (*4*) reported that smooth bromegrass hay contained 6.56 percent ash, 6.22 percent fat, 28.11 percent crude fiber, 13.60 percent protein, and 45.51 percent nitrogen-free extract. This hay was twice as rich in crude protein as timothy hay.

Nelson and Shepherd (*6*) observed that seeding a legume with smooth bromegrass prevents a "sod-bound" condition, and Rather and coworkers (*8*) observed that smooth bromegrass in mixtures with legumes speeds up the rate of curing of hay, and reduces danger of bloat when the mixtures are pastured.

Waldron and Porter (*12*) noted that at the North Dakota Station smooth bromegrass stays succulent longer than other grasses with the advent of warm weather.

PLAN OF THE INVESTIGATION

Samples of the grass were collected at approximately 2-week intervals and changes in chemical composition studied throughout the entire growing season. Samples of grass cut at a height of 4, 10, and 36 inches were fed to sheep in digestion stalls for the purpose of studying changes in the digestibility of the nutrients as the grass matured. The 36-inch growth coincided with the anther-falling stage, which ordinarily is that recommended as ideal in hay production.

Ten-day preliminary periods followed by 10-day experimental periods were employed in feeding each sample of forage. The equipment and detailed technique have been fully discussed elsewhere (*10*). The lambs were out of Lincoln × Merino ewes and sired by Hampshire rams.

DEVELOPMENT OF SMOOTH BROMEGRASS PLANTS

The rate of growth at various stages in the development of smooth bromegrass plants is shown in table 1. A moisture content of 72.38 percent at a height of 4.5 inches was reduced to 40.98 percent at the time the seeds were fully mature and the plants were 36 inches tall. The data on the composition of the dry matter in the forage samples is also summarized in table 1. The protein content of the early-spring samples was 13.61 percent, but this declined to 4.02 percent in the forage samples taken at the seed stage. As the protein content decreased the percentage of crude fiber increased from 19.77 to 35.92. The percentage of calcium declined during the season from 0.37 to 0.24 and the percentage of phosphorus declined from 0.32 to 0.13. Table 1 shows that the grass was quite nutritious up to the water stage of seed formation. There was no effective rainfall during the growing season of 1939; it was drier than any similar period in 46 years.

TABLE 1.—*Moisture content and chemical composition of smooth bromegrass clippings as related to height of plants and stage of maturity at approximately 2-week intervals during the growing season of 1939*

Date	Height of plants	Stage of matrity	Moisture content	Chemical composition on dry-weight basis						
				Protein	Crude fiber	N-free extract	Ether extract	Ash	Ca	P_2
	In.		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
May 3	4.5		72.38	13.61	19.77	53.41	3.73	9.48	0.37	0.32
17	10.0	Vegetative	73.53	13.26	21.76	52.44	3.40	9.14	.29	.32
31	16.5	½ headed	64.16	14.31						
June 15	24.0	Heads well formed	71.71	8.16	30.22	51.12	2.37	8.13	.25	.24
30	36.0	10 percent anthers	61.85	7.21	29.65	53.00	1.86	8.28	.30	.20
July 15	36.0	Water stage of seed	52.75	6.86	28.93	55.27	1.38	7.56	.24	.19
30	36.0	Seeds hard	43.93	5.10	29.84	54.40	1.80	8.86	.24	.18
Aug. 15	36.0	Seeds fully mature	40.98	4.02	35.92	52.00	1.59	6.47	.24	.13

EXPERIMENTAL RESULTS

The chemical composition of the grass clippings is shown in table 2. A complete summary of the number of lambs used, their weights, their dry matter intake, as well as coefficients of apparent digestibility for each nutrient as determined with individual lambs is presented in table 3.

Only three lambs were fed the 4-inch grass because of the large area required to supply forage at this stage. The dry-matter intake of lambs fed grass at the 4- and 10-inch stages is fairly comparable. At the mature stage the lambs consumed much less dry matter. With roughage rations (*10*) this inequality of dry-matter intake is not a disturbing factor in comparisons of coefficients of digestibility.

The average coefficients of apparent digestibility of the various nutrients are very nearly the same for the samples taken at the 4- and 10-inch stages. In this experiment smooth bromegrass up to the 10-inch stage retained its highly digestible character. A rather marked decrease in digestibility was noted at the hay stage. The grass 4 inches tall had a coefficient of 82 for its crude protein while at a 36-inch height the coefficient was 53. Crude fiber of the 4-inch grass was 85 percent digestible and at the 36-inch stage of growth only 56 percent digestible. A similar decline has been noted for the other nutrients.

TABLE 2.—*Chemical composition of smooth bromegrass at different stages of maturity, fed to lambs during digestion experiments*

Height of grass (inches)	Dry matter	Ash	Protein (N×6.25)	Crude fiber	N-free extract	Ether extract
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	37.20	10.67	14.65	20.81	49.35	4.52
10	37.48	7.50	12.06	28.31	48.93	3.20
36 [1]	85.45	8.43	6.23	35.55	47.45	2.34

[1] Anther-falling stage.

Table 3 also contains figures showing the percent of digestible crude protein content and the total digestible nutrient content of the fresh forage samples studied. By delaying grazing until the

10-inch stage, the nutritive qualities do not undergo much decline, while the carrying capacity of a given area is greatly increased through growth.

TABLE 3.—*Apparent digestibility of the nutrients in smooth bromegrass forage clipped at various stages of maturity and fed to lambs*

Height of grass (inches)	Lamb No.	Live weight of lambs		Daily dry-matter intake	Apparent digestibility					Total dry matter in forage	Digestible nutrients in fresh forage samples		
		Initial	Final		Dry matter	Crude protein	Crude fiber	N-free extract	Ether extract		Crude protein	Total	Nutritive ratio 1 to —
		Kilograms	*Kilograms*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
4	1	40.4	42.0	1,116	81	82	84	89	65				
	2	45.4	45.8	1,168	80	82	86	88	64				
	3	43.8	45.4	1,146	82	81	85	89	69				
Average					81.0	81.7	85.0	88.7	66.0	37.20	4.45	29.82	5.70
10	1	41.3	42.2	975	82	80	85	86	63				
	2	45.8	45.8	1,020	81	80	84	86	59				
	3	39.9	41.1	975	80	78	82	85	60				
	4	44.9	44.5	933	79	74	76	84	95				
	5	44.5	45.8	1,046	81	80	84	85	62				
	6	47.2	47.4	975	82	81	86	86	63				
Average					80.8	78.8	82.8	85.3	67.0	37.48	3.56	29.79	7.37
36 [1]	1	40.4	38.6	534	54	51	53	63	20				
	2	45.4	46.5	616	60	55	61	67	31				
	3	45.8	45.4	510	53	55	52	62	27				
	4	47.6	50.8	687	56	50	58	64	30				
	5	52.2	52.2	716	56	53	56	64	27				
	6	48.8	49.0	612	56	54	56	63	30				
Average					55.8	53.0	56.0	63.8	27.5	85.45	2.82	46.30	15.42

[1] Anther-falling stage.

In table 4 the digestible nutrient content of the dry matter of smooth bromegrass at three stages is contrasted with identical stages of crested wheatgrass as reported in a previous publication (*11*). A study of this table shows that the percentages of digestible nutrients in the dry matter of smooth bromegrass at the 4- and 10-inch stages are as high as in the common concentrates, like corn and barley. Furthermore, smooth bromegrass retains its high nutritive qualities to a more advanced stage of maturity than does crested wheatgrass, and at comparable stages it is a richer source of digestible crude protein and total digestible nutrients. Table 4 shows convincingly the superior nutritive qualities of smooth bromegrass over crested wheatgrass when these two grasses are produced under the soil and climatic conditions of the Palouse area of eastern Washington. However, each is peculiarly adapted to its own environment, smooth bromegrass to the more moist areas and crested wheatgrass to the drier areas. Crested wheatgrass has been highly praised in areas where smooth bromegrass cannot be grown successfully.

TABLE 4.—*Digestible nutrients in the dry matter of smooth brome-grass and of crested wheatgrass harvested at three stages of maturity*

Grass and height when clipped (inches)	Protein (N×6.25)	Crude fiber	N-free extract	Ether extract	Total digestible nutrients
Smooth bromegrass:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	11.97	17.69	43.77	2.98	80.14
10	9.50	23.44	41.74	2.14	79.50
36 [1]	3.30	19.91	30.27	.64	54.92
Crested wheatgrass (*11*):					
4	10.15	16.05	36.36	1.85	66.73
10	4.93	19.05	40.09	1.16	66.68
(1)	1.94	19.23	26.06	.34	48.00

[1] Anther-falling stage.

SUMMARY AND CONCLUSIONS

The protein content of the dry matter of smooth bromegrass clippings, taken at regular intervals throughout the growing season, decreased progressively from 13.61 percent to 4.02 percent. Crude fiber increased from 19.77 percent to 35.92 percent and plant height from 4.5 to 36 inches. The calcium content declined from 0.37 to 0.24 percent and phosphorus from 0.32 to 0.13 percent during the same interval.

By delaying the grazing of smooth bromegrass until it is 10 inches tall, the nutritive qualities do not decline and the carrying capacity appears to be greatly increased. Bromegrass up to a height of 10 inches retains its highly digestible character. The dry matter was 81 percent digestible at the 4- and 10-inch stages and only 55.8 percent digestible at the 36-inch stage. Crude protein, crude fiber, and nitrogen-free extract followed similar trends. Smooth bromegrass retains its high nutritive qualities to a more advanced stage of maturity than does crested wheatgrass and at identical stages of growth is superior to it in digestible crude protein and total digestible nutrients.

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U. S. GOVERNMENT PRINTING OFFICE: 1941